Can Myxosporean parasites compromise fish and amphibian reproduction?

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Research into fish and amphibian reproduction has increased exponentially in recent years owing to the expansion of the aquaculture industry, the need to recover fishery populations, the impact of endocrine disruptors on the aquatic environment and the global decline of amphibian populations. This review focuses on a group of parasites, the Myxozoa, that affect fish and amphibian reproduction. Lists of the myxosporeans that specifically infect gonads are provided. Most of these are parasitic of freshwater hosts, and most amphibian cases are reported from testes. Sex specificity and sex reversal are discussed in relation to gonadal parasitism. The immune response of the fish to the infection is described, and the contribution of the immunoprivilege of gonads to host invasion is emphasized. The pathological effect of these parasites can be significant, especially in aquacultured broodstocks, on some occasions, leading to parasitic castration. Although myxosporean parasites are currently not very frequent in gonads, their impact could increase in the future owing to the transactions in the global market. Their easy release into the aquatic environment with spawning could make their spreading even more feasible. In the absence of commercial drugs or vaccines to treat and prevent these infections, there is an urgent need to develop specific, rapid and reliable diagnostic tools to control and manage animal movements. In addition, much effort is still to be made on deciphering the life cycle of these organisms, their invasion strategies and their immune evasion mechanisms.

Keywords: Myxozoa; pathology; immune response; gonads; sex

1. WHY MYXOSPOREA AND REPRODUCTION?

Research into fish reproduction has increased exponentially in recent years owing to the parallel expansion of the aquaculture industry, the need to recover fishery populations and the impact of endocrine disruptors on the aquatic environment (Mills & Chichester 2005; Powell et al. 2005). The interest in amphibian reproduction has also increased because of the global decline of wild populations (Souder 2000; Pough 2007). Nevertheless, the knowledge of the effect of pathogens, and particularly that of parasites, on fish and amphibian reproduction is scarce and scattered through different disciplines and fields of expertise. In fishes, most investigations have focused on the effect of parasites on reproductive behaviour, sexual ornamentation and the relationship with immunocompetence (Barber et al. 2000; Skarstein et al. 2001; Ottowá et al. 2005), and much attention has been paid to the impact of some digeneans on the reproductive success of declining frog populations (reviewed by Johnson et al. (2004)). Collective data suggest that the Myxozoa could emerge as a serious pathological threat for the reproduction of these aquatic organisms.

The myxozoan clade comprises more than 2180 species, most of which are fish parasites, and infect any tissue and host organ (Lom & Dyková 2006). In recent years, the Myxozoa have received extensive attention owing to their pathological impact on both fisheries and aquaculture (Alvarez-Pellitero & Sitja-Bobadilla 1993; Moran et al. 1999; Rigos et al. 1999; Palenzuela 2006), but also for the still intriguing aspects of their life cycle and controversial taxonomy (Kent et al. 2001; Canning & Okamura 2004; Jiménez-Guri et al. 2007). This review will focus on those myxosporeans that affect fish and amphibian reproduction, owing to the pathological effect that some of them have on aquacultured broodstocks, and their detrimental effect on the reproductive success in wild stocks. Some aspects related to their transmission and host–parasite relationship will receive special attention.

Parasites can affect fish reproduction either directly or indirectly, depending on the target organ. This review will concentrate on the first case, when the parasite is present in the gonad. Nevertheless, worthy of mention among non-gonadal infections is the case of the multivalvulid Kudoa paniformis. This parasite negatively affects the reproductive effort of the Pacific hake (Merluccius productus), with the decrease of its fecundity being proportional to the intensity of infection. Thus, a female with a muscle parasite load of 340 pseudocysts per gram has only 10 per cent of the fecundity of non-infected fish (Alderstein & Dorn 1998). As with this case, the exact mechanisms involved in the alteration of fish reproduction by non-gonadal parasites have not yet been elucidated. Several possibilities have been suggested: (i) the parasite extracts energy and nutrients from the host, which are not

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destined to reproductive effort and (ii) the parasite induces physiological, immunological or ethological changes in the host, which impair mating, gonad maturation or larval survival.

The incidence of parasites in fish and amphibian gonads is difficult to assess since gonads are not always examined in parasite monitoring. Nevertheless, in recent years, more attention has been paid to gonads owing to the impact of endocrine disruptors in aquatic animals (Blazer 2002) and to the world amphibian decline (Pasmans et al. 2006). Despite their huge diversity and vast host number, myxosporeans have not been described in gonads as frequently as in other organs. A list of species specifically affecting the gonads of fish and amphibians is presented in tables S1 and S2 of the electronic supplementary material, respectively. Most are parasites of freshwater hosts, as only *Ceratomyxa auberbachii*, *Kuda ovicora* and *Sphaerospora testicularis* have been reported from marine fish. It is also noteworthy that most are not only specific of gonads, but also of a particular sex (§5). For amphibians, all the known records concern *Myxobolus* species and infect anurans, since there is only one unpublished finding of a myxosporean in the testes of an imported salamander (*Ambystoma maculatum*) kept in a German pet shop (F. Mutschmann 2004, 2007, personal communication).

Tables S1 and S2 do not include species that have been reported in other organs as well as in gonads, or others which are systemic and gonads are just another site. This is the case of *Kuda ovatai* found in the ovarian stroma, in addition to other organs of gilthead sea bream (*Sparus aurata*) (figure 1a) (Diamant et al. 2005). *Myxobolus diaphanus* in Fundulus diaphanus (Cone & Easy 2005) and *Sphaerospora lucioperca* in *Stizostedion lucioperca* (Moshu 1992) are cases of ovarian infection in addition to other fish organs. In European sea bass (*Dicentrarchus labrax*), *Sphaerospora dicentrarchi*, a systemic histozoic parasite (Sitja-Bobadilla & Alvarez-Pellitero 1992a), can be found in the testicular stroma, within the connective tissue (figure 1b,c) and also in the seminiferous lumen, when the normal architecture of the testicular cyst has been disrupted by *S. testicularis* in mixed infections (figure 1d). Among amphibians, a *Myxobolus* sp. was found to infect systemically 19 of 25 *Bufo regularis* imported from Egypt, including gonads (testes and ovaries), which were completely destroyed by the parasite (figure 1e,f) (F. Mutschmann 2004, 2007, personal communication).

### 2. MACROSCOPIC AND ANATOMICAL LESIONS

Gonad infections do not generally produce external signs until the intensity of infection is high. European sea bass infected with *S. testicularis* during the spawning season show abdominal distension because of the accumulation of ascites or gonad hypertrophy. The genital pore may also appear enlarged and reddish. Testes can sometimes be hyaline or yellowish with haemorrhagic foci (figure 2a–c). At the end of this period, infected testes appear necrotic, hardened and much larger than non-infected ones (figure 2d,e). Tilapia ovaries infected with *Myxobolus dahomeyensis* appear with whitish nodules consisting of suppurating thick liquid visible through the membrane, which contains a large amount of spores (Gbankoto et al. 2001). In some other cases, if normal gonad development is impaired, part of the abdomen appears sunken. In addition, *Clarias gariepinus*-infected ovaries exhibited whitish macroscopic nodules corresponding to *Myxobolus gariepinus* plasmodia (Reed et al. 2003), and ovaries infected by *Myxobolus kamijae* are enlarged, whitish and with soft consistency (Obiekezie & Okaeme 1990). In any case, the external appearance of the fish is altered, and its commercial value is decreased or it may even be rejected by consumers. Obviously, if these fish are part of a broodstock, they are no longer useful as breeders.

In amphibians, no gross pathology was found to be associated with the myxosporean testicular infection with *Myxobolus fallax* (Browne et al. 2002), *Myxobolus bufonis* (Upton et al. 1992) or *Myxobolus* sp. (Théodoridès et al. 1981). However, the testes infected with *Myxobolus chimbuenis* may appear enlarged and slightly discoloured (Ewers 1973), and frogs heavily parasitized with *Myxobolus hyla* appear sick and emaciated, with their testes swollen (Johnston & Bancroft 1918) or filling almost all the abdominal cavity (Browne et al. 2002). In addition, testes of *Bufo marinus* heavily infected by *M. bufonis* exhibit whitish irregular nodules of different sizes, which correspond to masses of the parasite (F. Mutschmann 2004, 2007, personal communication) (figure 2f).

### 3. PATHOLOGY

Gonad infection can lead to parasitic castration, depending on the exact location and intensity of infection. Parasites located in the ovarian or testicular stroma are less pathogenic than those located within oocytes or seminiferous tubules. Examples of the first situation are *Henneguya testicularis* (Azvedo et al. 1997), *Myxobolus testicularis* (Tajdari et al. 2005), *Myxobolus paranensis* (Bonetto & Pignalberi 1965), *Myxobolus algonquensis* or *Sphaerospora ovophila* (Xiao & Desser 1997). A mixed case is *Myxobilus* sp. in the three-spined stickleback (*Gasterosteus aculeatus*), which invades both the ovarian stroma and the seminiferous tubules. In both sexes, heavy infections may lead to parasitic castration, as infected tubules contained few or no spermatids or spermatozoa and the parasite almost entirely replaced the contents of the ovaries. The impact of this myxosporean on the host population dynamics is unknown, but an effect on the host reproductive success is likely (Longshaw et al. 2007) (figure 3a,b). *Henneguya amazonica* is an intermediate case. It massively infects the ovarian stroma, but can also enter the oocyte, lying between the zona pellucida and the follicular epithelium, which leaves the inner part of the oocyte free of the infection and may be fertilized normally (Torres et al. 1994).

When parasites are located in the germinal tissue, both the destruction of the already mature gonad and the inhibition of normal gonad development have been reported. Some examples follow. The development of *M. dahomeyensis* in tilapia ovaries leads to the formation of cavities and the destruction of the oocytes. The ovary wall maintains its integrity, but the proliferation of the parasite inside the oocyte leads to its exhaustion and the final disintegration of its membrane (Gbankoto et al. 2001) (figure 3c). *Myxobolus kamijae* also invades mature oocytes (Obiekezie & Okaeme 1990). *Kuda ovicora* infects the ovary of Caribbean fishes belonging to *Labridae* (figure 3d,e). Its plasmodia and spores develop inside oocytes, within the internal oocyte layer.
and the vitelline granules, which are gradually reduced until oocytes are fully occupied by parasitic stages, and attain even larger dimensions than healthy oocytes. The final result is that the reproductive effort is largely reduced as infected oocytes, if fertilized, do not divide, whereas the energy investment in reproduction is the same as in non-parasitized fishes (Swearer & Robertson 1999).

In European sea bass, *S. testicularis* invokes parasitic castration because of the destruction of both testicular germinal cells and Sertoli cells that are essential for normal spermatogenesis (Nóbrega et al. 2009). The infection starts with a few trophozoites in the lumen of seminiferous tubules (figure 3g). At this stage, diagnosis of the infection with a fresh drop of seminal fluid is fairly difficult (figure 3f). In the next step, the parasite proliferates in the lumen, feeding on spermatozoa (Sitja-Bobadilla & Alvarez-Pellitero 1993a) and blocking spermatogenesis in the testicular cysts. Transmission electron microscopic observations have shown that Sertoli cells are damaged, and therefore their normal phagocytic function, essential at the end of the reproductive season, and their function as nutritional and architectural support to spermatogonia in the testicular cysts are altered (Sitja-Bobadilla & Alvarez-Pellitero 1993b). In a further step, the lumen is completely occupied by parasitic stages, the germinal tissue is almost completely destroyed.

**Figure 1.** Non-specific infections of fishes and amphibian gonads. (a) *Kudoa iwatai* in gilthead sea bream ovary. Masses of spores are indicated by asterisk. (b–d) *Sphaerospora dicentrarchi* in European sea bass testis: (b) typical bag-like group of spores in the connective tissue; (c) mature spores (arrows) embedded in a pigmented cell accumulation in the interstitial tissue during the spawning season; (d) spore (arrow) in a fish also infected with *S. testicularis* (arrowheads). (e,f) *Myxobolus* sp. in *B. regularis* testis. Stainings of histological sections: (a) haematoxylin–eosin; (b–d) toluidine blue; (e) Ziehl–Ngelsen; (f) fresh smear. Scale bar, (a,c,e) 20 μm; (b,d,f) 10 μm. Histological material of (a) was provided by Dr A. Diamant (NCM, Israel); (e,f) illustrations provided by Dr F. Mutschmann (Exomed, Germany).
and only a few spermatogonia are left (figure 3f). The infection is thus easy to detect in fresh smears of seminal fluid, which appears very fluid and yellowish (figure 3h). In the final stages of the invasion, testicular cysts are disrupted, the myxosporean spreads beyond testes, and ascites appears in the abdominal cavity.

In amphibians, the information on the pathology is very scarce, but collective data also point towards the damage of the reproductive capacity. *Litoria darlingtoni* infected by *M. chimbuensis* exhibited very few sperm and little meiotic activity (Ewers 1973), and the plasmodia of *Myxobolus* sp. occupied almost all the testis of the Egyptian toad *B. regularis*, causing atrophy of the testicular tissue, necrosis of the tubular cells, destruction of the seminiferous tubules and extensive haemorrhages (Mubarak & Abed 2001). Other myxosporeans not yet identified to the species level caused destruction of the gonads in several amphibian species imported to Germany from different tropical countries (F. Mutschmann 2004, 2007, personal communication) (figure 4a–f).

4. HOST IMMUNE REACTION

Gonads are a special site when it comes to the immune response, as some parts are isolated from the immune system. This means that they could become a paradise for invaders and also the Achilles’ heel of the immune system. The immunoprivilege of the male gonad was thought to lie exclusively on the blood–testis barrier (BTB), maintained by Sertoli cell functions. This physical barrier between the general circulation and testicular tissue stops the pass of exogenous micro-organisms to the seminiferous tubules, conceals testicular antigens from the immune systems and prevents effector cell access. The main reason for this barrier is to avoid the reaction of the immune system against gametes (haploid), which are considered allogenic for the host (diploid), avoiding autoimmune reactions (Mochida & Takahashi 1993). Damage to reproductive organs would pose a threat to the survival of the species. Nevertheless, recent research in mammals has revealed that the immunotolerance of the testis is established and maintained not only by the BTB, but also by immunological factors (Fijak & Meinhardt 2006).

This would explain the absence of cellular reaction against parasites while they are located in the lumen, as happens with *S. testicularis*. However, when the testicular cysts are disrupted, and the infection spreads beyond testes, leucocytic infiltration consisting of granulocytes and macrophages is evident. At the end of the spawning season, most infected tubular lumen is occupied by encapsulated parasites or parasitic debris, with a strong fibrotic host reaction. These capsules can become highly pigmented, and granuloma can even remain until the next spawning season (figure 3j). Granulocyte and pigmented cell accumulations are abundant close to the capsules and probably constitute accumulations of phagocytic cells, cell debris and oxidized lipids (Blazer 2002). In the three-spined stickleback, an increased thickening of the interstitial tissue and a fibrotic reaction around some tubules were also apparent in testes infected by *Myxobilatus* sp. However, in light infections, there was a limited host response (Longshaw et al. 2007). Parasite encapsulation is a host reaction that aims to isolate the parasite and to prevent its dispersal, as described in several myxosporoses. However, in *S. testicularis* infections, the efficacy of these fibrotic capsules is questionable because they can prevent the parasite spreading with seminal fluid, but they also result in the accumulation of residual material in the germinal tissue, even up to the next spawning season. Therefore, these ‘scars’ of the past infections can reduce spermatogenesis in the future.

In ovaries, parasites also evade host immune reaction while located inside oocytes. They provide a very suitable place for the development of the parasite since they are full of nutritive substances. Thus far, no host immune reaction against myxosporean infecting oocytes has been reported.

No information is available on the immune response of amphibians against myxosporeans.

5. SEX SPECIFICITY AND SEX REVERSAL

The number of myxosporean parasites reported in fish ovaries is higher than that in testes, but most amphibian records are from testes (tables S1 and S2 in the electronic supplementary material). Generally, the reasons for this sex specificity are unknown, although several possibilities can be hypothesized: (i) specific nutritional requirements: oocytes and spermatozoa have clear differences in composition and size and therefore offer a different type

Figure 2. Gross lesions produced by *S. testicularis* in European sea bass during (a–c) and after (d, e) the spawning season. (a) Slight infection with haemorrhagic foci (arrowhead); (b) strong infection with hardened yellowish appearance and visible nodules (arrowheads); (c) hyalinized margins (arrowhead) in a strongly infected testis; (d) massive necrosis in sea bass testis (arrow) (picture courtesy of Carlos Zarza, Skreting, Spain) and (e) detail of the necrosis. (f) Massive infection of *B. maculatum* testis with *M. bufonis* (picture by F. Mutschmann, Exomed).
Figure 3. Pathological effects of myxosporeans in fish gonads. (a,b) Myxobilatus sp. in Gasterosteus aculeatus, note the scarce oocytes left in the ovary (a, arrows) and a detail of spores in the ovaric stroma (b, arrow). (c) Scanning electron microscopic image of a tilapia ovary with oocytes destroyed by M. dahomeyensis. (d,e) Kudoa ovivora from the ovary of T. bifasciatum: (d) detail of a spore with scanning electron microscopy; (e) fresh smear, phase contrast. (f–j) Sphaerospora testicularis from European sea bass: fresh smears of seminal fluid with slight (f, Nomarski) and heavy (h, phase contrast) infections; sections of testis with slight (g, trophozoites (arrows) are visible in the seminiferous lumen) and massive infections (i) during and after the spawning period (j). Note the seminiferous tubuli completely occupied by parasitic stages (asterisk in i), the thickened interstitial tissue (i) and the granulomata with parasite debris (asterisk in j). Histological sections stained with (a,b) haematoxylin–eosin or (g,i,j) toluidine blue. Scale bar, (a,c) 1 mm; (b,f–h) 10 μm; (j) 20 μm; (e) 25 μm; (i) 125 μm. Illustrations courtesy of Dr M. Longshaw (CEFAS, UK) (a,b), Dr A. Marques (University of Montpellier II, France) (c) and Dr S. E. Swearer (University of Melbourne, Australia) (d,e).
of nutrients to the parasites; (ii) particular ways of transmission and host–parasite coevolution; and (iii) immunological differences among host sexes. In birds and mammals, the highest prevalence of infection of certain parasites in males has been attributed to the immunosuppressive effect of testosterone (Zuk 1996). In fact, sex ratio and social stressors such as courtship and fighting for females can also alter immune responsiveness (Binuramesh et al. 2006). For S. testicularis, a trophic hypothesis could be postulated, as trophozoites develop within the seminal fluid, feeding on spermatozoa, and ovocytes are obviously much larger, and the ovarian environment is clearly different. The timing of the infection seems to be related to this fact, as immature sea bass were never found infected, and the parasite proliferates in synchronization with spawning (Sitjà-Bobadilla & Alvarez-Pellitero 1993c). Similarly, in infections by M. dahomeyensis, immature oocytes are unaffected (Gbankoto et al. 2001).

In gonochoristic fish species, sex reversal is an abnormal situation and has been suggested as a marker of exposure to oestrogenic substances in the aquatic environment (Jobling et al. 1998). In sea bass, the presence of ovarian tissue in the testes of non-parasitized males (ovo-testis) has been described (Blázquez et al. 1999). However, a higher percentage of ovo-testis is found in males infected by S. testicularis than in uninfected ones under the same conditions (A. Sitjà-Bobadilla 1991, unpublished data). In these cases, previtellogenic oocytes are scattered through the testicular tissue, even inside the tubular lumen, and

Figure 4. Pathological effects of myxosporeans in amphibian gonads. (a,b) Myxobolus sp. in Hyperolius concolour testis. (c,d) Myxobolus sp. in Litoria caerulea ovary. (e,f) Myxobolus sp. in Litoria infrarufa. Masses of parasite stages are indicated with asterisk. Histological sections stained with (a,c,e,f) haematoxylin–eosin. Scale bar, (a,d) 1 mm; (e) 200 μm; (b,d,f) 10 μm. Pictures courtesy of Dr F. Mutschmann (Exomed) (a–d) and Dr P. Zwart (Utrecht University, The Netherlands) (e,f).
surrounded by spermatozoa. This phenomenon could be owing to a hormonal dysfunction invoked by the destruction of Sertoli cells, since these cells have been described to have steroidogenic functions in several fish species (Nóbrega et al. 2009).

In sequential hermaphrodite fishes, a possible parasitic influence on the sex allocation of its host has been postulated for the protogynous Thalassoma bifasciatum. Thus, the data suggest that females infected by K. ovivora not only have a lower reproductive success, but also change sex earlier and at a smaller size than uninfected ones (Schärer & Vizoso 2003). This host–parasite system has implications for the field of evolutionary ecology. Sex change can be viewed as ‘host death’ from the point of view of the parasite, as the host tissue in which the parasite sporulates disappears, potentially leading to conflicts of interest between host and parasite over sex change (Schärer & Vizoso 2003).

6. TRANSMISSION

The life-cycle pattern of Myxosporea entails the alternation of a vertebrate host and definitive one (an invertebrate). Thus, spores released from fishes are infective for the involved invertebrate, but the generality of this life cycle has not yet been established. In fact, fish-to-fish transmission has been demonstrated for members of the genus Enteromyxum (Diamant 1997; Redondo et al. 2005). The final consequence of gonad infection is the release of the parasite with gametes during spawning, which favours its transmission to the environment and, therefore, to the next putative host. If invertebrate hosts were not involved, then horizontal or vertical transmission could happen. The first has been described for some parasites with holocercan cycles, such as microsporeans and coccidians. Among fish myxosporeans, it has been proposed for K. ovivora since infected oocytes have distinctive features that favour their ingestion by hosts (Swearer & Robertson 1999), and the epidemiological data of S. testicularis suggest that fish-to-fish transmission might take place (Sitjà-Bobadilla & Alvarez-Pellitero 1993), but this has not been demonstrated experimentally. In amphibians, testicular infection by Myxobolus spp. may favour sexual transmission of spores during spawning, allowing spore deposition in aquatic environments, where invertebrate stages may be encountered, and perhaps subsequent infection of tadpoles may occur (Browne et al. 2002). The shedding of M. fallax spores has been demonstrated to be sexually mediated through reproductive hormones without affecting fertility (Browne et al. 2006).

Vertical transmission (to the next generation) can happen when gametes are intracellularly infected (uniparental, transovarial), or when the parasites accompany gametes during fertilization and enter the embryo rapidly (biparental, perinatal) (figure 5). This has been described for microsporean parasites of invertebrates and vertebrates, including fishes (Summerfelt & Warner 1970).
It is suspected to be the case of *H. amazonica*, as infected oocytes seem to be viable, and there is a possibility that they may proceed with normal development, fertilization and survive hatching (Torres et al. 1994).

### 7. CONCLUSIONS AND FUTURE PERSPECTIVES FOR RESEARCH

The effect of some myxosporean parasites on the reproduction of their hosts is considerable, leading to parasitic castration in some occasions. In testicular infections, the immunoprivilege of gonads contributes to the invasion of the host. Although myxosporean parasites are not currently very frequent in gonads, their impact could increase in the future owing to the transactions in the global market. Thus, the trade of fishes, their gametes and embryos as a result of fish farming and the need for re-stocking wild populations and the ornamental fish market, together with the movements of amphibians for food consumption, pet shops and biomedical research, exert a high risk for the transmission and spread of gonadal myxosporan parasites. The likely fish-to-fish transmission of some of these and their easy release into the aquatic environment with spawning could make their spreading even more feasible. As for Myxozoa in general, there is an urgent need to find effective treatments for these gonadal myxosporoses, since only experimental trials have been performed for *S. testicularis* (Sitjà-Bobadilla & Alvarez-Pellitero 1992b). In the absence of useful parasiticides and vaccines, control measures can only be based on preventive and good management measures of the broodstocks. Thus, the development of specific, rapid and reliable diagnostic tools to control and manage animal movements is crucial. In addition, much effort is still to be made on deciphering the life cycle of these organisms, their invasion strategies and immune evasion mechanisms. Furthermore, we are still largely in the dark concerning the endocrine disruption made by gonadal parasites and the evolution of host–parasite systems to balance the host’s (to reproduce) and parasite’s (to grow on reproductive organs) interests.

Part of the information on sea bass sparerosporosis was obtained in research projects funded by Spanish Ministerio de Educación, Cultura y Deporte MAR-98/1000 and the Spanish Ministerio de Ciencia y Tecnología (project no. AGL-2002-0475-C02-01). The author is in debt to Dr A. Diamant (Israel), Dr A. Marques (France), Dr A. Diamant (Israel), Dr A. Marques (France) and Dr P. Zwart (The Netherlands). The author is in debt to the Spanish Ministerio de Ciencia y Tecnología (project no. AGL-2002-0475-C02-01). The author is in debt to Dr A. Diamant (Israel), Dr A. Marques (France), Dr A. Diamant (Israel) and Dr P. Zwart (The Netherlands), for their contributions providing histological material or illustrations.

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