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NOTE

Spontaneous adenocarcinoma of the gas gland in *Nothobranchius* fishes

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ABSTRACT: *Nothobranchius* fishes (Cyprinodontiformes), known for their genetically encoded extremely compressed lifespan, are considered an excellent vertebrate model for the research of aging. Unlike the rapid accumulation of data concerning their biology, ecology and genome, knowledge of their age-related diseases, including tumours, is still very limited. This Note reports spontaneous neoplastic lesions in the swim bladder gas glands of *Nothobranchius furzeri*, *N. kadleci* and *N. orthonotus*. Based on light and transmission electron microscopy, the neoplastic proliferation of gas gland cells was classified as adenocarcinoma. There was a concurrent proliferation of haemopoietic cells in the kidney interstitium in all individuals diagnosed with this type of primary neoplasia.

KEY WORDS: *Nothobranchius* fishes · Spontaneous neoplasia · Gas gland adenocarcinoma

1. INTRODUCTION

The occurrence of tumours in fish has been a subject of review since the middle of the last century (Schlumberger & Lucké 1948, Wellings 1969, Mawdesley-Thomas 1971, 1975). Contributions to a milestone symposium on fish pathology (Ribelin & Migaki 1975) and chapters published thereafter in textbooks on fish diseases provide insight into the history of knowledge development and the growing interest in tumours of poikilothermic vertebrates. Morphological comparison of tumours in fish and higher vertebrates, virus aetiology of fish neoplasias, the type and incidence of fish tumours related to chemical contaminants of the water environment and carcinogenicity tests with small fish species have interested countless authors. A clearly formulated vision that fish tumour studies may contribute to a better understanding of the general mechanisms of cancer development was published by Masahito et al. (1988). Recently, the zebrafish *Danio rerio* has been instrumental in increasing knowledge about fish tumours. An analysis of the zebrafish model for toxicology and pathology, based on almost 600 papers (Spitsbergen & Kent 2003), together with a more recent review of neoplasia and neoplasm-associated lesions in laboratory colonies of zebrafish (Spitsbergen et al. 2012), have revealed that more data is available on the experimental induction of specific zebrafish cancer types than on spontaneous tumour incidences. Although the zebrafish is considered the premier and leading non-mammalian vertebrate model system for understanding genes and signalling pathways that control the development and mechanisms of diseases including cancer, annual killifish are emerging as an alternative suitable model system (Cellerino et al.

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2016, Platzer & Englert 2016, Polačik et al. 2016, Dodzian et al. 2018, Hu & Brunet 2018). The evolutionarily encoded short lifespan of annual fishes of the genus *Nothobranchius* represents a great research advantage in the study of age-related spontaneous tumours. To date, spontaneous neoplastic proliferations have been described in the kidney and liver of *Nothobranchius furzeri*, *N. guentheri, N. kadleci* and *N. korthausae* (Cooper et al. 1983, Di Cicco et al. 2011, Baumgart et al. 2015, Blažek et al. 2017). Limited information exists about potential target organs other than the liver and kidney. Here we describe neoplastic lesions of the swim bladder gas gland found in *Nothobranchius* fishes reared under laboratory conditions.

2. MATERIALS AND METHODS

Spontaneous histopathological changes were examined in a total of 70 laboratory-bred individuals, including 55 *Nothobranchius furzeri*, 12 *N. orthonotus* and 3 *N. kadleci*, all with noticeable behavioural impairments and clinical disease. Prior to dissection, fish were euthanized with an overdose of anaesthetic (clove oil). The standard sampling procedure consisted of fast and careful removal of the left side body wall followed by fixation of the body cavity organs and kidney *in situ* or in individual partial samples. In preparation for light and electron microscopy examination, size of the fish and the character of any grossly visible lesions determined whether the entire viscera was processed by routine paraffin technique or small samples from individual organs were fixed with 2% osmium tetroxide, dehydrated in acetone dilution series and embedded into Spurr resin for examination of semithin and ultrathin sections under light and transmission electron microscopes, respectively.

3. RESULTS

3.1. Clinical signs and gross lesions

The most noticeable clinical signs observed in *Nothobranchius* fishes housed in laboratory aquaria were lethargy, difficulty or inability to swim in the water column, recumbency along the bottom of the aquarium and respiratory distress. Gross lesions included epidermal haemorrhages and occasional cutaneous ulcers along the ventral abdominal wall. Internal lesions most commonly included renomegaly, hepatomegaly and splenomegaly. Less frequent but conspicuous were lesions in enlarged swim bladders. Contrary to the minute gas gland in the transparent thin-walled swim bladders of healthy individuals, the swim bladder wall in the clinically impaired individuals was diffusely thickened, the lumen filled with semiliquid dark material and small gas bubbles, and the gas gland enlarged forming a prominent red lens-like structure.

3.2. Light and electron microscopy

In 22 (16 *Nothobranchius furzeri*, 4 *N. orthonotus* and 2 *N. kadleci*) out of the 70 individuals evaluated, proliferating haemopoietic cells were found in the kidney, spleen and liver. Surprisingly, the same type cells were also present in the content of the swim bladder. In 5 (3 *N. furzeri*, 1 *N. kadleci*, 1 *N. orthonotus*) out of the 22 fish with abnormal swim bladder content (Fig. 1) there was a neoplastic proliferation of gas gland cells associated with the capillary network (rete mirabile) (Fig. 2a,b). In these swim bladders, the high rate of gas gland proliferation was best seen in longitudinal sections (Fig. 2c).

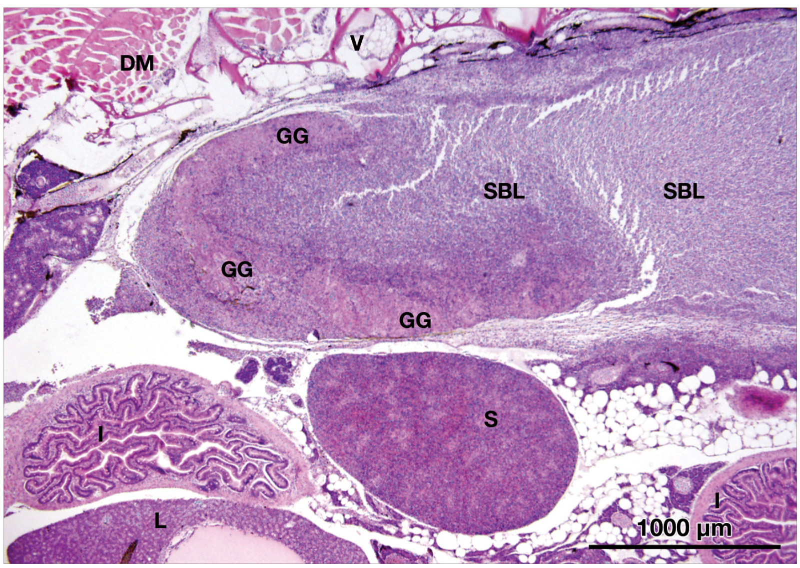

Fig. 1. Sagittal section through body cavity of *Nothobranchius furzeri* illustrating the relative positions of internal organs including swim bladder with altered gas gland. DM: dorsal muscles; GG: gas gland; I: intestine; L: liver; S: spleen; SBL: swim bladder lumen with cellular content; V: vertebrae

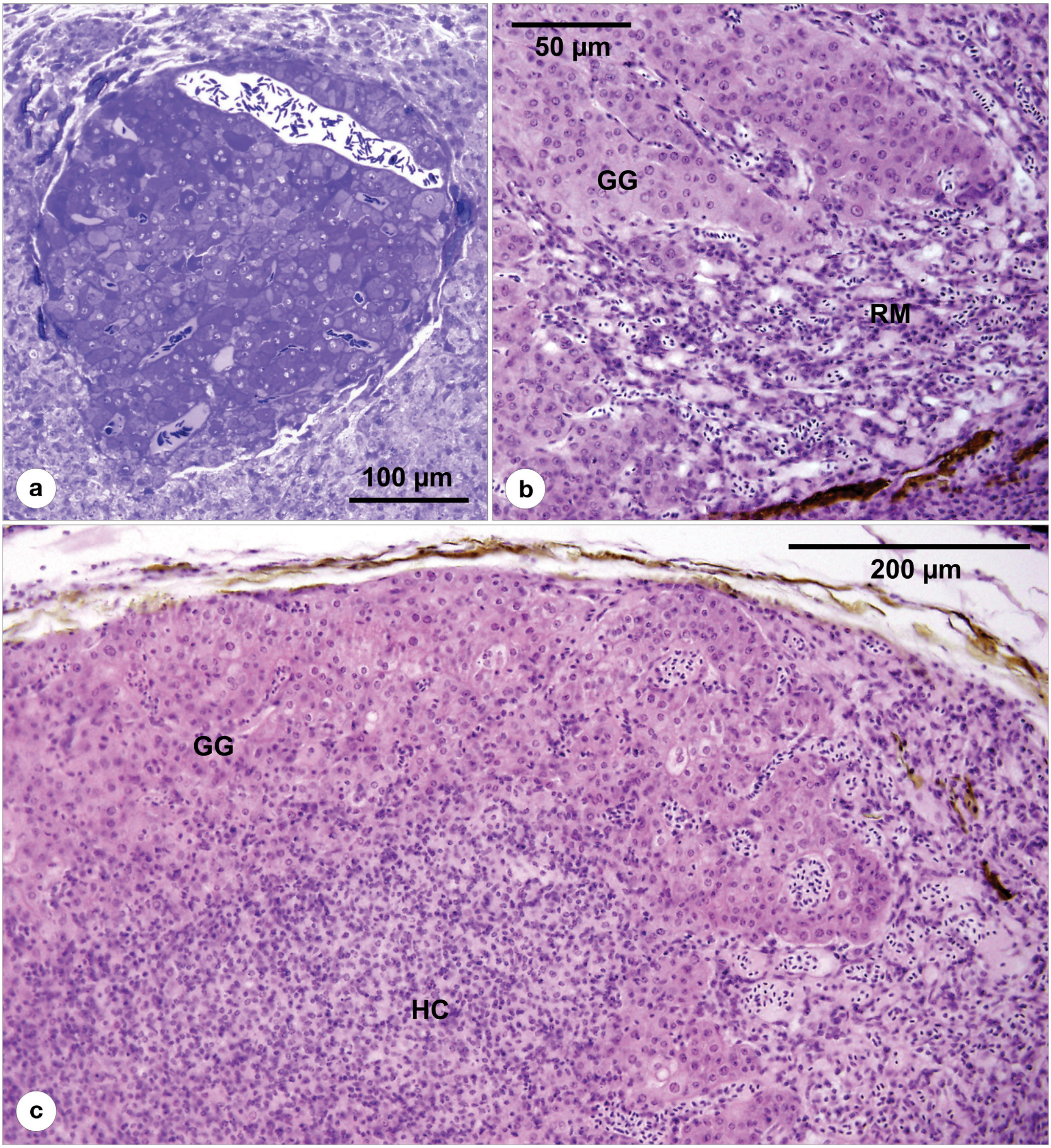

Fig. 2. Light microscopy of neoplastic proliferation of gas glands in swim bladders of *Nothobranchius* fishes. (a) Gas gland seen in tangential semithin section stained with toluidin blue. (b) Gas gland cells associated with capillaries of the rete mirabile. (c) Longitudinal section through a swim bladder of *Nothobranchius furzeri* affected by dual neoplastic proliferation. Neoplastic gas gland cells concentrated in the upper region pervade the swim bladder content (below) which is rich in haemoblasts. (b,c) Histological sections stained with H&E. GG: gas gland cells; RM: rete mirabile; HC: haemoblasts

The contents of these swim bladders were pervaded by masses of proliferating gas gland cells with variable size and stainability (Fig. 3). Cytomegalic gland cells with pale cytoplasm and multinucleation were seen in both paraffin and semithin (resin) sections. Fourteen mitotic figures (Fig. 3c) were counted

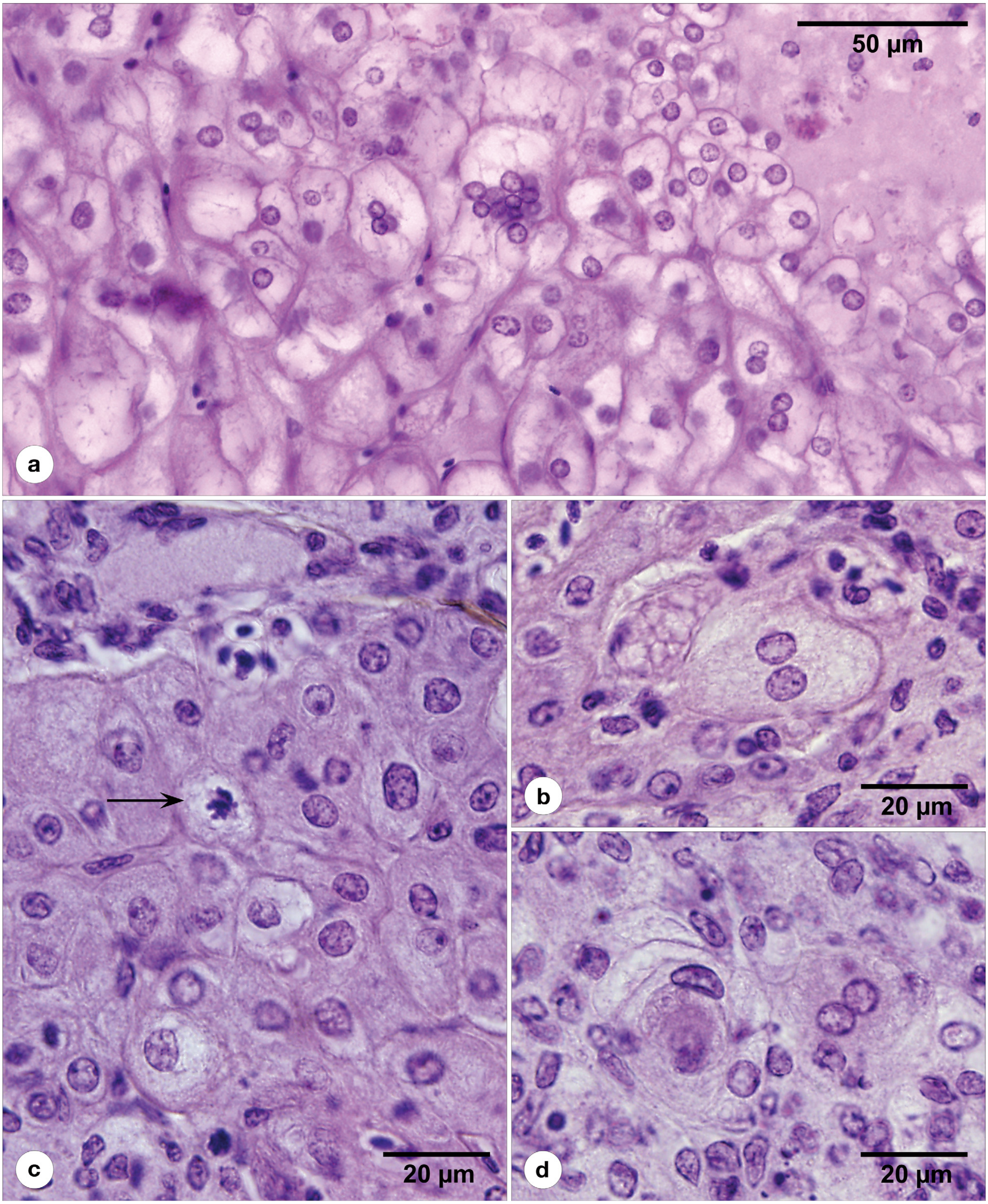

Fig. 3. Neoplastic proliferation of gas gland cells in swim bladders of *Nothobranchius furzeri* as seen at the cellular level (H&E staining). (a) Overview of proliferating gas gland cells differing in size, number of nuclei per cell and cytoplasm density. (b) Binucleate gas gland cell with pale cytoplasm. (c) Neoplastic gas gland cells with relatively dense cytoplasm; note the mitotic figure (arrow). (d) Two phagocytosed neoplastic gas gland cells within the content of a swim bladder

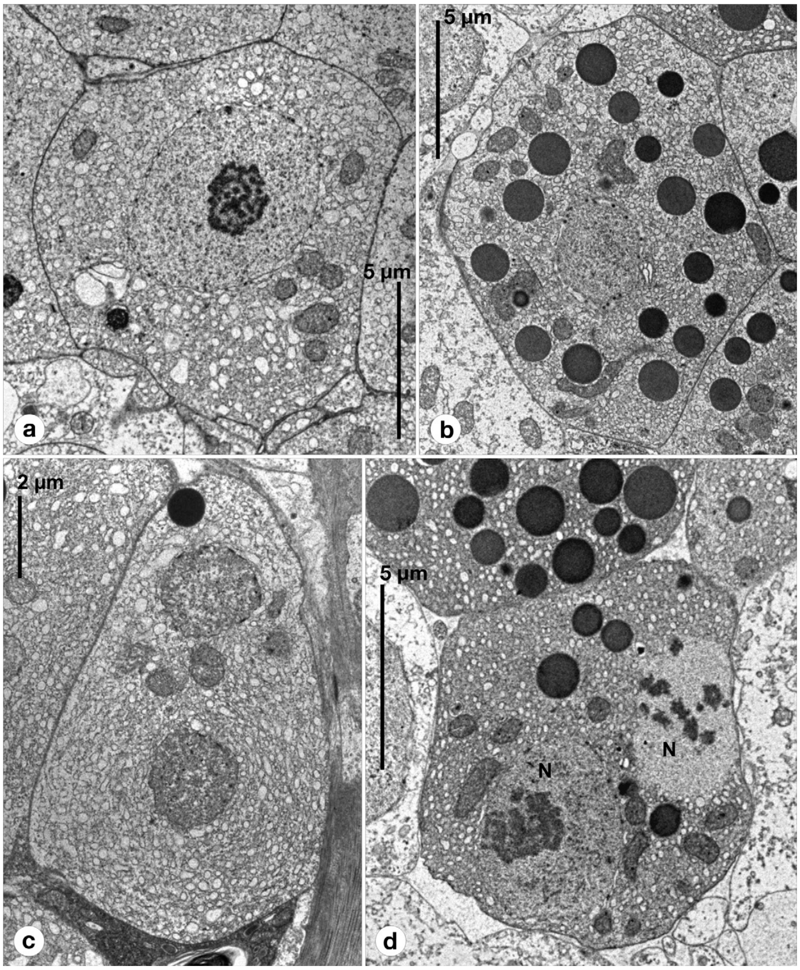

Fig. 4. Ultrastructure of gas gland cells proliferating in swim bladders of *Nothobranchius furzeri*. (a) A cell which resembles those in an unaffected gas gland. (b) Gas gland cell with several mitochondria, numerous small vacuoles, and conspicuous electron-dense bodies resembling lipid droplets. (c) Binucleate gas gland cell with signs of anaplasia or regressive changes. (d) Mitotically active gas gland cell with 2 nuclei (N)

in 10 representative fields of vision at high magnification. Transmission electron microscopy supplemented the light microscopy findings with fine details. The proliferating gas gland cells contained numerous tiny vacuoles/vesicles, large electron-dense (intensely osmiophilic) bodies in the cytoplasmic matrix and highly euchromatic nuclei (Fig. 4). Multiple nuclei were found in different stages of the mitotic cycle (Fig. 4d). Proliferating cells with relatively well-preserved fine structure contained numerous mitochondria; however, their cristae were quite pleomorphic. Along with the prevailing cells that resembled those of healthy glandular tissue, numerous cells displaying anaplastic or severe regressive changes were seen in all cases. The neoplastic proliferation of gas gland epithelium in *Nothobranchius* meets most of the standard criteria for histological diagnosis of adenocarcinoma.

4. DISCUSSION

Within the wide range of target organs and types of spontaneous tumours described in fish, the swim bladder and specifically gas gland are rarely mentioned. Haemangioma was the first and, for a long time, only known tumour reported from the swim bladder in codfish (Johnstone 1925). Much later, Stolk (1957) described adenoma in the swim bladder of the guppy *Poecilia reticulata*. The histology of the latter tumour was only documented with line drawings, nonetheless, the essential details match those recognized in our *Nothobranchius* fishes. In medaka *Oryzias latipes* and guppy used as untreated control fish in carcinogenicity tests, Fournie et al. (1999) diagnosed 3 types of proliferative lesions that arose from the gas gland epithelium: hyperplasia (in 1 guppy), adenoma (in 2 medakas and 1 guppy) and adenocarcinoma (in 6 guppies). The large adenomas from the medaka were of a solid growth pattern, while that from the guppy was characterized by a tubular pattern of growth. Evaluation of the adenocarcinomas found in guppies revealed variation in the general pattern, total size and proportion of the necrotic areas. The gas gland tumours in guppies and those found in this study differed mainly in that those in guppies were described and documented as solid tumours, whereas in *Nothobranchius*, the neoplastic proliferation was dual, with the gas gland carcinoma cells permeating the mass of haemopoietic cells. The haemopoietic cell proliferation predominating in the kidney, a concurrent problem noted in the cases of *Nothobranchius* gas gland neoplasia, deserves more detailed study based on a larger assemblage of fish and a well scheduled age-related progressional sampling. The histopathology of 5 *Nothobranchius* individuals with both types of proliferative lesions in the swim bladder cannot provide details on the sequence of pathological changes, their interaction or the hypothetical promoter of carcinoma development. However, responding to the important call from the key papers on spontaneous and induced fish neoplasias (Spitsbergen et al. 2009, 2012), any new information is important.

The histopathological examination of clinically diseased *Nothobranchius* has partially clarified the phenomenon of adult 'belly-sliders', well known to killifish hobbyists for years. Our findings of irreparable proliferative changes that impair the function of the swim bladder in subadult and adult fish supplement the previous explanation of this clinical status due to an insufficient inflation of the swim bladder in the appropriate post-hatching time (Podrabsky 1999).

Our study has shown that the histology of the swim bladder in *Nothobranchius* fishes should be elaborated in more detail, as analogy with data on the swim bladders of eel, perch, ruffe, *Alcolapia* and *Fundulus* (Copeland 1969, Jasiński & Kilarski 1969, Maina 2000) does not provide complete information. We propose that especially the topography of the gas-secreting and gas-resorbing epithelium of the luminal surface of the swim bladder of *Nothobranchius* should be further investigated.

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