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Original Research

Pathogens and parasites of the black-striped pipefish (*Syngnathus abaster*) from the Tunis North Lake, Tunisia

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Abstract
The black-striped pipefish, *Syngnathus abaster* member of the Syngnathidae family, is a small species occurring in either brackish or fresh waters associated with vegetated habitats, few data are known about the anomalies that encounter the species in coastal lagoons and estuaries. A survey of *S. abaster* from the Tunis north lagoon (Tunisia), where the species occurs and breeds among vegetation, was conducted during 2007-2011, a multitude of anomalies were observed in external bodies and guts of some specimens of *S.abaster* and were suspected to be related to parasitic infections. The parasitic analysis were made in 2011 and revealed the presence of a number of visible cysts in the epidermis, musculature and intestine. A focussed histological study of the fish revealed the presence of microsporidian infections in the epidermis, musculature and the intestine. In addition, a coccidian infection of the intestine and a systemic digenean infection were noted. Pathology associated with the myotrophic microsporidian consisted mainly of pressure atrophy and disruption of affected muscles, whilst the gut-dwelling microsporidian infection may have affect absorption rates. Other infections were considered relatively benign. The results are discussed in relation to the general ecology of the host. In conclusion, it is suggested in this study to assess the health status of *S. abaster* in the lagoon using more data about the impact of such infections on the fish behaviour and to study the transmission of the parasites in this area.

INTRODUCTION

A variety of infectious organisms are known to affect fish behaviour and health and are used as biomarkers of the relative health of an ecosystem. Few species (nematodes, trematodes, cestodes, acanthocephalans) may be transmitted to humans from marine fish and are capable of infecting them [1-4]. The use of fish parasites has relevant application to fisheries management and to assess the safety of marine products [5].

Members of the Syngnathidae have been recorded as hosts for bacteria, protistans, microsporidians, myxozoans, monogeneans, digeneans, cestodes, nematodes and copepods [6-14].

The black-striped pipefish, *Syngnathus abaster* is a small euryhaline species, commonly associated with vegetated habitats in the Mediterranean, Black and Caspian Seas and the Atlantic Coast up to the Bay of Biscay [15,16]. It has also become established in freshwater biomes like as the River Danube and in the Ukraine [17]. It has little commercial value but is an important component of sea grass ecosystems. The investigation of non commercial fish health, inhabiting estuaries is important in order to understand their role in disease transmission to commercial stocks [10]. Ben Alaya *et al* [18] have investigated morphological abnormalities in *S. abaster* and Sárria *et al* [19] considered the impact of endocrine disrupting chemicals on behaviour and early life history of *S. abaster* juveniles. The black-striped pipefish sampled in the Lower Danube was found to be infected with 8 species of metazoans, including digeneans, cestodes,

nematodes and acanthocephalans which were considered to have been acquired in its new environment [20]. No data was provided on the overall impact of these infections on its health. In its natural range, *S. abaster* has been recorded as a host for several digeneans and ciliates [20], the parasitic copepod *Ergasilus ponticus* [21] and the acanthocephalan *Acanthocephaloides irregularis* [22].

The current study describes the histopathology of infections in wild caught *S. abaster* from Tunis North Lake, Tunisia, which were originally examined as part of a study on the ecology of this species. This paper provides the first record of a microsporidian infection in *Syngnathus abaster* with large cysts either externally under the skin or with numerous smaller cysts adhered to the intestinal wall.

MATERIALS AND METHODS

The study was carried out in the Tunis North Lake (36°49'52.66"N; 10°15'27.21" E), a shallow water plane of 2,600 hectares located in the North East of Tunisia between the town of Tunis and the Gulf of Tunis and connected to the Mediterranean Sea. *Syngnathus abaster* resides throughout the year in areas of the lagoon where vegetation (*Ruppia cirrhosa*, *Chaetomorpha linum*, *Enteromorpha* sp., *Caulerpa* sp., *Zostera sp*.) occurs. Sampling activity was performed in the northern part of the Tunis lagoon between 2007 and 2011 for a biological and diet study of the species. Specimens of *S. abaster* were caught using a drag net (2 mm mesh size). Total length (TL, mm) was measured. The parasitic study was conducted in 2011. Fish with external dermal cysts were photographed and preserved. 120 individuals of *S.abaster* were dissected for a biological and gut content study. Guts with apparent cysts were preserved for histological analysis. Gills and liver were examined and reserved for further histological examination.

Fish with external dermal and gut cysts were submitted to the Cefas laboratory in Weymouth, England. Tissues were decalcified in 10% formic acid for up to 4 days then rinsed in 70% industrial methylated spirits and stored until processing for histology. Fish were processed in a vacuum infiltration tissue processor then embedded in wax blocks according to standard procedures. Sections were cut at approximately 3µm then stained with either haematoxylin and eosin (H&E) or Giemsa and examined using a Nikon E800 (Nikon UK Ltd, Kinston Upon Thames, UK). Representative images were captured using the LUCIA™ screen measurement system (Laboratory Imaging Ltd, Praha, Czech Republic).

RESULTS

Fish lengths ranged from 80 to 115 mm. From the specimens of *S.abaster* observed, 5 of them showed externally visible dermal cysts manifesting as white raised lumps covered with a thin layer of epithelium (Fig. 1). Internal cysts were noted on the intestinal walls of 6 individuals. The microsporidian infection occurring in the musculature was characterised by the formation of large xenomas (Fig. 1), often taking up over 25% of a cross-sectional view of pipefish (Fig. 2). The xenomas were surrounded by several layers of fibroblasts and epithelial fragments. Furthermore, several xenomas were frequently seen co-occurring in the same area. Compression of the surrounding musculature was not seen although distension of the skin epithelium to accommodate the subcutaneous cysts was noted. No breaches of the epithelium were noted in the fish examined. The microsporidian infection of the intestine and visceral organs was characterised by the formation of small white xenomas measuring approximately 2-5 mm in diameter (Fig. 3). The xenomas appeared to be surrounded by a host-derived fibrous membrane (Fig. 4). Host response was generally limited.

Fig. 1. Low power view of external surface of pipefish (*Syngnathus abaster*) showing numerous large cysts (arrow).

A minor infections with small digeneans metacercariae were observed in the gills, liver and muscle of 9 pipefishes from those with severe microsporidian infection by several xenomas in the musculature and intestine. One case of coccidian infection was found in the gut of 1 specimen.

There was a fibrous encapsulation of the digeneans noted in the gills, liver and musculature (Fig. 5) and in one individual, a melanised reaction was recorded surrounding digeneans in the musculature. The low level coccidian infection of the gut did not elicit a host

response and was limited to early gamont stages occurring in the epithelial cells (Fig. 6).

Fig. 2. Histological section through trunk muscle of pipefish with large myotrophic microsporidian xenomas (arrow) apparent that fills a large proportion of the muscle block. Giemsa stain.

Fig. 3. Low power view of intestine comprising of a large number of microsporidian xenomas.

DISCUSSION

The histological study revealed a microsporidian infection with large xenomas in the skeletal musculature of 5 individuals and a microsporidian infection of the intestine in 6 individuals. There have been few reports of parasites and pathogens of members of the Syngnathidae and these are often limited to descriptions of specific parasite groups or of disease outbreaks under aquarium conditions [11,23,24]. Few studies have involved the study of wild caught individuals (9,10,12,18,25].

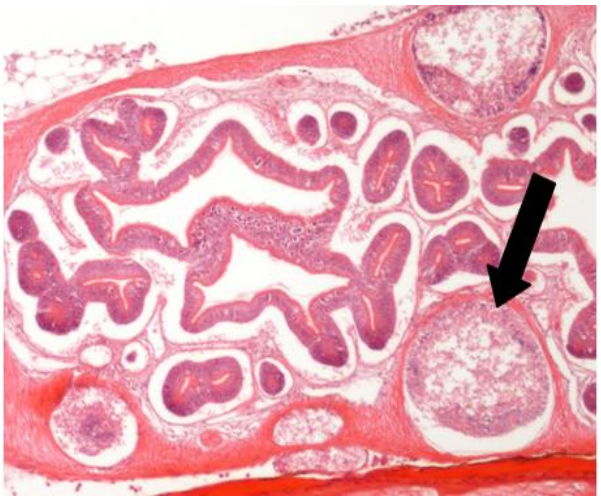

Fig. 4. Histological section through intestine of *S. abaster* with numerous microsporidian cysts visible (arrow). Pathological response restricted to a localised host response (H&E).

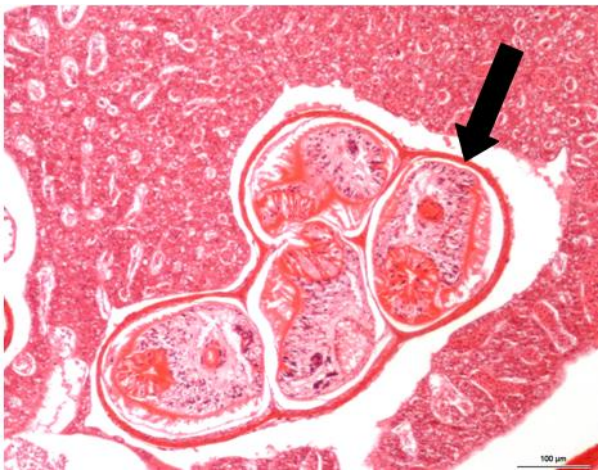

Fig. 5. Encysted digeneans in the liver of *S. abaster* surrounded by a thin membrane (arrow). (H&E).

Of greatest concern during the current study was the presence of the microsporidian infection in the musculature and viscera. Microsporidians are obligate, intracellular fungi of a wide range of hosts [26] and are considered to be highly specialised fungi [27, 28, 29]. Blasiola [1] described *Glugea heraldi* from the subcutaneous tissues of the seahorse *Hippocampus erectus*. A second microsporidian, *Glugea acuta* has been noted in the connective tissue of the dorsal fin muscle of *Syngnathus acus* in the Atlantic and Mediterranean coasts of France. The apparent strict tissue specificity has not been widely commented on and it has been suggested that the microsporidian needs to be redescribed as it has not apparently been recorded

since its original discovery. Specific samples for ultrastructural and molecular studies are required to accurately identify the parasite and should form the basis of future work, in particular given its high intensity in the host.

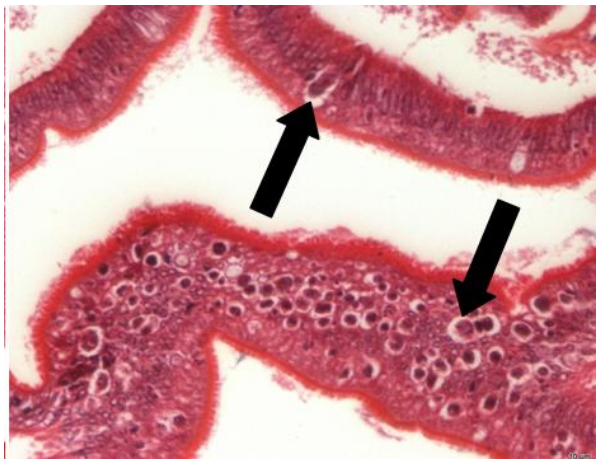

Fig. 6. Early developmental stages of an unidentified coccidian in the intestinal epithelium cells of *S. abaster.* (H&E).

In the present work, it is probable that the muscle infection is detrimental to pipefish survival. It infects a large swath of the musculature with complete loss of the muscle structure. Infected pipefish are likely to have difficulty swimming which will in turn limit their ability to avoid predators and to capture prey items. Rosenqvist and Johansson [30] suggested that male pipefish discriminated against females infected with the digenean *Cryptocotyle lingua* which manifested as black spots on the skin of the fish. Whilst it was not tested in the current study, it would be interesting to determine if the presence of the large, visible subcutaneous cysts associated with the microsporidian infection altered the sexual behaviours of pipefish during courtship.

The presence of intestinal microsporidian infection throughout the gut is likely to have a detrimental effect on food absorption, particularly given the large number of cysts noted and its intimate association with the absorptive gut epithelium. Although *S. abaster* has no market or economic value and is not a major prey item, it is an important component of sea grass communities. Sária *et al* [19] considered that the species could be a model species for assessment of contaminant effects, particularly in estuarine environments, as it has a long lifespan ensuring bioaccumulation of any contaminants and it spends a large proportion of its time in estuaries. Estuarine environments can be major sinks for chemical pollutants, some of which will be hazardous for living organisms [31]. Thus, examination of some infections in *S.abaster* from estuaries may be useful as biological indicators of proxies of the health status of the environment. During the current study, however, no toxicopathic lesions were detected in the fish examined.

Longshaw *et al* [10] suggested that examination of non-commercial species was useful in providing additional data on the role, if any, in disease transmission between different trophic levels in the environment. A lack of data on disease conditions may affect breeding success of pipefish in capture situations [32, 33]. In addition, knowledge of the health status of *S. abaster* may prove useful in understanding why *S. abaster* has successfully invaded new habitats. Whether *S. abaster* are good exemplars of the enemy release hypothesis requires further investigation [34]. Future studies should further characterise the parasites noted in the current study, assess further the impact of such infections on mating behaviour and determine if infections of *S. abaster* are widespread in those areas that they habituate.

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