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Tetramicra brevifilum (Matthews & Matthews, 1980) (Microsporida: Tetramicriidae) in a new fish host, *Lophius budegassa* (Spinola, 1807) in Spain

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Abstract *Tetramicra brevifilum*, a microsporidian parasite of *Scophthalmus maximus*, was found in *Lophius budegassa* for the first time. This parasite was detected in 5 of 199 hosts captured in the coastal waters of Barcelona (Northwest Mediterranean), which enlarges the geographic distribution of this microsporidian. Affected fish did not show any external sign of disease, and cysts of *T. brevifilum* were found associated with the body musculature but were easily differentiated from those of *Spraguea lophii*, another microsporidian present in this host. A case of simultaneous infection by both *T. brevifilum* and *S. lophii* was found.

Introduction

Many species of microsporidia have been described that parasitize a great variety of fish (Canning and Lom 1986; Lom and Dyková 1992). Some of them cause serious diseases affecting various tissues and organs and might be responsible for extensive mortality both in nature and in aquaculture (Canning and Lom 1986). *Tetramicra* (Microsporidia: Tetramicriidae) is a genus of microsporidia consisting of a single species, *T. brevifilum,* described by Matthews and Matthews (1980). It affects 10% of the wild populations of *Scophthalmus maximus* (L., 1758) (Pleuronectiformes, Scophthalmidae) off the northern coast of Cornwall (Matthews and Matthews 1980). It has also been reported in turbot fish farms along the coast of Galicia (Atlantic coast of Northwest Spain), where it causes high levels of mortality (Estévez et al. 1992; Figueras et al. 1992). During routine examinations of the black anglerfish *Lophius budegassa* (Spinola, 1807) (Lophiiformes: Lophiidae) from the coast of Barcelona (Mediterranean coast of NE Spain) and during a survey for the microsporidia *Spraguea lophii* (Doflein 1898; Weissenberg 1976) (Microsporidia: Sprague idae), a different species of microsporidia was observed. The characteristics were the same as those described for *T. brevifilum*.

We report the presence of *T. brevifilum* in a new host (black anglerfish) for the first time as well as extension of the geographic distribution of this parasite to the Northwest region of the Mediterranean sea. The only species of microsporidia thus for described in black anglerfish has been *S. lophii* (Canning and Lom 1986; Lom and Dyková 1992; Sprague et al. 1992). Furthermore the simultaneous presence of the two parasites in the same individual is described.

Materials and methods

During April 1996 we studied 199 individuals of *Lophius budegassa*. These fish had been caught off the coast of Barcelona (Northwest Mediterranean). Each fish was measured, weighed, dissected, and examined for the presence of parasites. The microsporidian cysts found were either observed with a light microscope or fixed with 2.5% (v/v) glutaraldehyde in 0.1 *M* cacodylate buffer (pH 7.34) for 2 h at 4 °C and postfixed with 1% (v/v) OsO_4 in the same buffer for 1 h at 4 °C. After fixation the material was dehydrated in acetone and embedded in Spurr's resin (Spurr 1969). Ultrathin sections of 60 nm were obtained with a Reichert Ultracut ultramicrotome and then stained with uranyl acetate and lead citrate (Reynolds 1963) for observation in a Philips EM301 transmission electron microscope operating at 80 or 100 kV.

Results

The presence of *Tetramicra brevifilum* was observed in 5 of the 199 (2.51%) black anglers studied during April 1996. The average length of the infected fish was 26 cm and the average weight was 198.9 g. The infected fish did

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not show external signs of disease. During dissection and after removal of the skin for a detailed examination of the muscular tissue, some whitish, oval (1–3 mm in diameter) nodules were observed (Fig. 1). Some of these cysts were associated with the conjunctive layer on the muscle surface, whereas others could be seen within the muscular tissue of the tail. Observation of the cysts in a squash under a light microscope clearly showed the presence of typical microsporidian spores (Fig. 3).

The degree of infestation by *T. brevifilum* was rather variable. One of the infected fish presented a generalized infection involving dozens of cysts all over its body. The other four fish showed only a few cysts associated with caudal muscles. No cyst was observed in other organs. Muscular tissue did not show signs of degeneration, even in the case of generalized infection. In fresh tissue the spores were ovoid, being wider toward the posterior end, and measured 3.7 (± 0.03) $\times$ 2.7 μm (± 0.02). A large posterior vacuole containing a conspicuous inclusion was present (Fig. 3). TEM observation of spores revealed that the polar filament had four turns (in some cases, three). The spores had a single nucleus and also presented large electron-dense inclusions in the cytoplasm as well as an inclusion in the posterior vacuole (Fig. 4). These are typical characteristics of *T. brevifilum* as described by Matthews and Matthews (1980). Polyribosomes formed a paracrystalline pattern associated with the polar filament of the spore (Fig. 9).

Of the 199 black anglers studied, 22 (11.06%) were infected by the microsporidian *Spraguea lophii*. The fish with a generalized infection of *T. brevifilum* was also infected by *S. lophii* in a cranial nerve, although the infection by this second microsporidian was very mild. In view of the double infection, a χ^2 test was performed to assess the independence of the infections. The test was clearly significant ($\chi^2 = 5.8 \times 10^{-3}, \varepsilon = 0.05$), confirming the independence of the presence of *T. brevifilum* and *S. lophii* in a single individual of *L. budegassa*. No other individual black angler infected by *T. brevifilum* was observed in later surveys during 1996.

Discussion

This is the first report of *Tetramicra brevifilum* in a host other than *Scophthalmus maximus*, in which it was first described. The area of distribution of this parasite is described for the first time as having enlarged to the West coast of the Mediterranean sea – to date it has been reported only in the North Atlantic (Matthews and Matthews 1980; Estévez et al. 1992; Figueras et al. 1992; Dyková and Figueras 1994).

Spraguea lophii is a well-known parasite of the genus *Lophius* (Doflein 1898; Priebe 1971; Weissenberg 1976; Loubès et al. 1979; Amigó et al. 1993). The cysts of this microsporidian are easily distinguishable; they are usually associated with the central nervous system, despite their also being found frequently on the peripheral nervous system. They produce large grape-shaped masses of cysts with a diameter of up to 2 cm (Fig. 2). Sometimes, cysts of *S. lophii* are also found associated with the spinal marrow innervation. We have rarely observed fish with generalized infections of *S. lophii* presenting with cysts associated with the nervous plexus of different organs (heart, digestive system, liver). The cysts on these organs usually are isolated and do not form grape-shaped groups. Nevertheless, *S. lophii* cysts

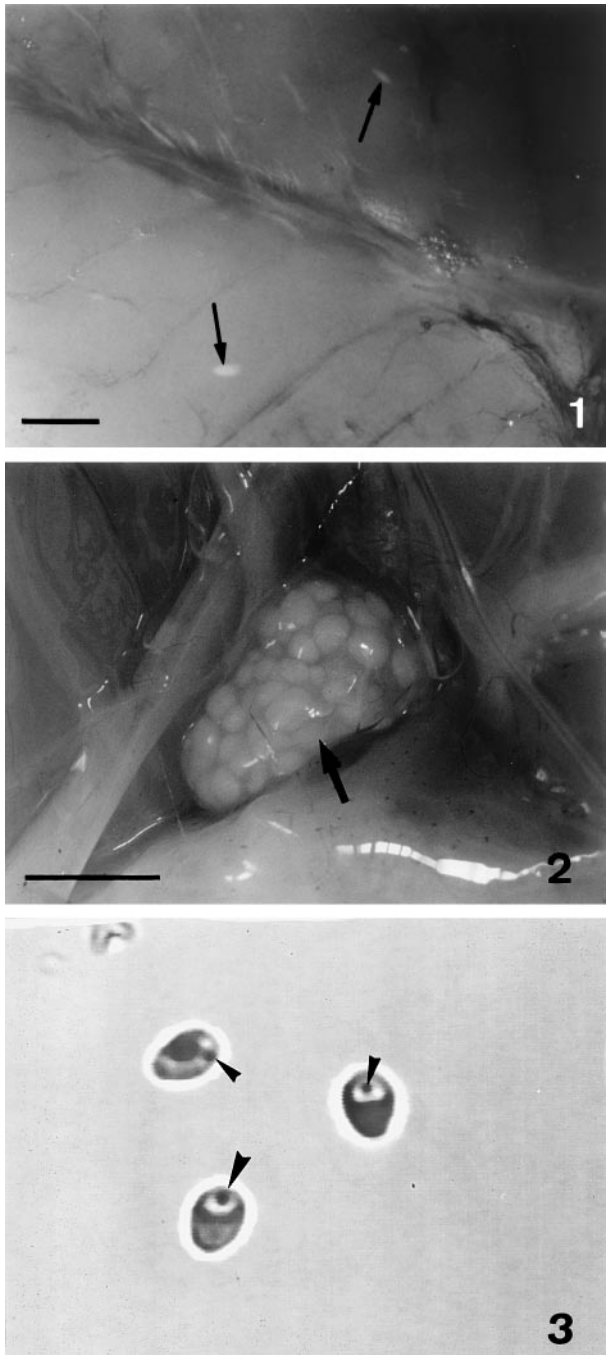

Fig. 1 Whitish *Tetramicra brevifilum* foci (*arrows*) in fresh musculature of *Lophius budegassa*. *Bar* 1 cm. **Fig. 2** Cluster of cysts of *Spraguea lophii* (*arrow*) infecting ganglion cells of *L. budegassa*. *Bar* 1 cm. **Fig. 3** Fresh spores of *T. brevifilum*. Note the inclusion within the posterior vacuole (*arrowheads*). ×1800

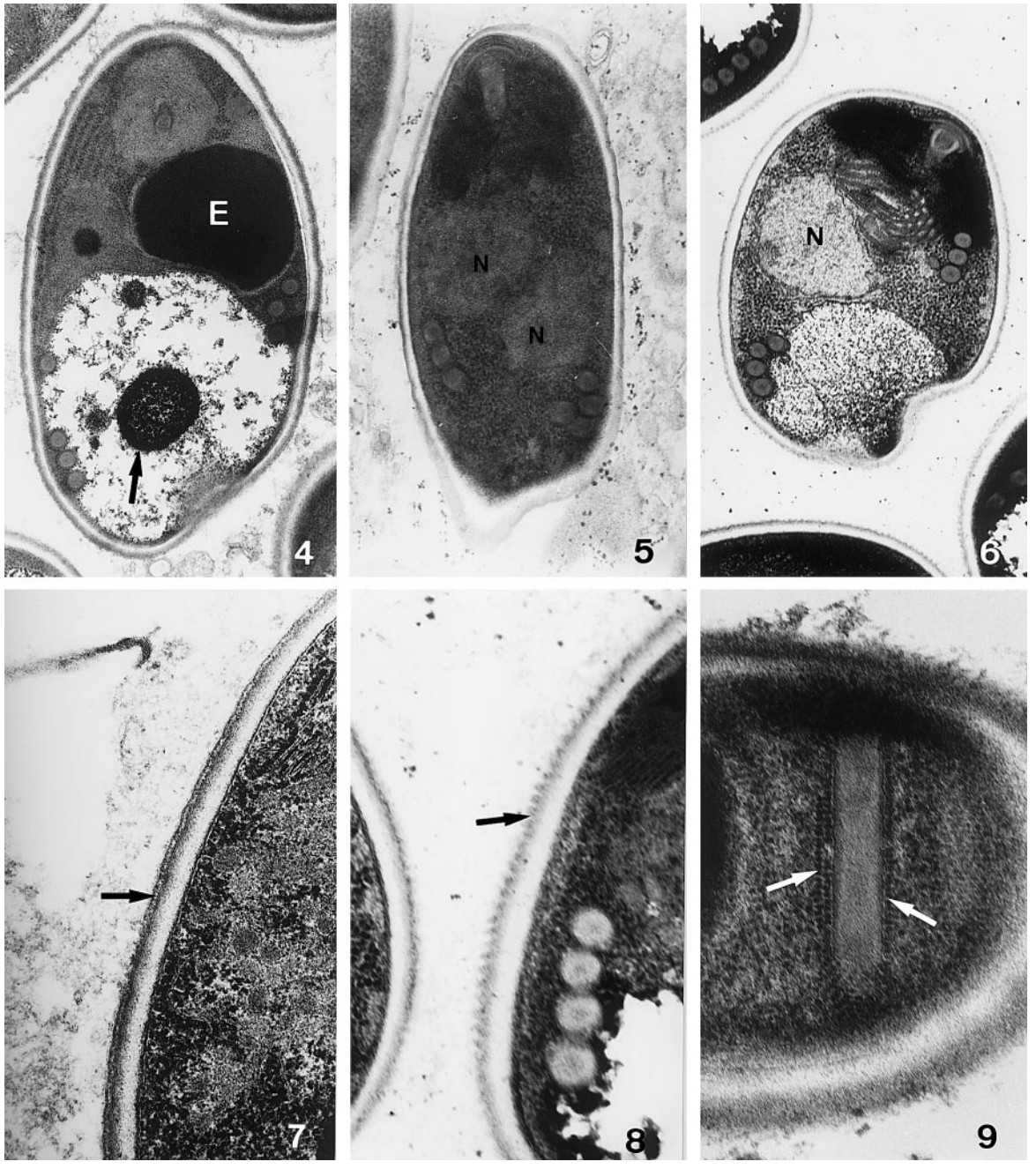

Fig. 4 Transmission electron micrograph (TEM) of a *T. brevifilum* spore. Note the large electron-dense inclusion body (*E*) and the inclusion within the posterior vacuole (*arrow*). ×23,000. **Fig. 5** TEM of a diplokaryotic spore of *S. lophii*. Two nuclei (*N*) closely arranged and forming a diplokaryon can be observed. ×23,000. **Fig. 6** TEM of a monokaryotic spore of *S. lophii*. A single nucleus (*N*) is visible. ×20,000. **Fig. 7** Detail showing the smooth exospore surface (*arrow*) characteristic of spores of *T. brevifilum*. ×77,100. **Fig. 8** Detail showing the fine ridges of the exospore (*arrow*) that are characteristic of monokaryotic spores of *S. lophii*. ×77,000. **Fig. 9** Polyribosomes arranged into a paracrystalline pattern around the polar tube in the spore of *T. brevifilum* (*arrows*). ×80,000

appear differently from *T. brevifilum* cysts; they are clearly spherical and are never immersed in the muscle tissue mass. *S. lophii* cysts have two types of spores of differing size and morphology [*Nosemoides*-type oval spores (Fig. 6) and *Nosema*-type spores (Fig. 5), which are thinner and slightly curved]. On the other hand, *T. brevifilum* has one only type of spore. This is easily distinguishable from *S. lophii* spores by light microscopy or electron microscopy (Table 1).

Figueras et al. (1992) observed polyribosomes in a paracrystalline pattern associated with the polar tube of immature sporoblast tubes of *T. brevifilum*, and these authors stated that "The presence of polyribosomes in the immature tubes, if confirmed in other populations of *Tetramicra brevifilum*, can be used as an additional differential feature. Polyribosomal structures were found in many other species (e.g., *Heterosporis finki*, Schubert

Table 1 Characters differentiating *Tetramicra brevifilum* spores from *Spraguea lophii* spores

	T. brevifilum	*S. lophii* (monokaryotic)	*S. lophii* (diplokaryotic)
Spore size	3.7 × 2.7 μm	4.2 × 2.5 μm	3.7 × 1.4 μm
Spore shape	Oval	Ovoid	Curved
Diplokaryon presence	No	No	Yes
Posterior vacuole inclusions	Yes	No	No
Electrodense accumulations in cytoplasm	Yes	No	No
Exospore	Plain (Fig. 7)	Ornamented (Fig. 8)	Plain

1969); however, they were not associated with the polar tube.'' Our observations confirm the presence of such polyribosomic structures related to the polar tube (Fig. 9). However, we do not believe that this feature should be used as a differential trait of *T. brevifilum*, since polyribosomic structures have been found in many other species, also in association with the polar tube [e.g., *Nosemoides syaciumi*. (Faye 1992) and *Microgemma ovoidea* (Amigó et al. 1996a)].

The size of fresh *T. brevifilum* spores found on *L. budegassa* differs significantly both from that provided in the original description (4.8 × 2 μm; Matthews and Matthews 1980) and from data reported by Estévez et al. (1992; 4.25 × 2 μm). We know that in normal hosts under ordinary conditions the spores are rather uniform in shape and size (Weiser 1977). However, it is well known that microsporidians that affect various species of fish can present different spore sizes, depending on the host (e.g., *Glugea anomala*, *G. stephani*; Lom and Dyková 1992). Thus, the differences found may be attributed to the observation that the parasite was found in an atypical host. Furthermore, *T. brevifilum* itself has shown some variations in size within the same host (Estévez et al. 1992). Therefore, a proposal to establish a new species as based substantially on the spore size and slight shape differences would not be justifiable in view of the natural variability of microsporidians.

The finding of one individual simultaneously infected by two species of microsporidian is unusual, although not exceptional, since this has previously been described in insects [e.g., *Nosema lunatum* and *Thelohania californica* on *Culex tarsalis* (Kellen et al. 1967); *N. heterosporum* and *T. nana* on *Plodia interpunctella* (Kellen and Lindegren 1969)] and in humans [*Enterocytozoon bieneusi* and *Septata intestinalis* (Franzen et al. 1996)].

It is generally accepted that microsporidian infections are transmitted by direct ingestion of the spores. The spores can be present in water or in the sediment as a result of defecation and urination by previous hosts (Kramer 1976). Cannibalism has also been reported as a pathway of transmission for Microsporidia (Matthews and Matthews 1980; Baxa-Antonio et al. 1992). Parasite transmission could also occur after host death, followed by liberation of the spores either directly into the water after decomposition or through the digestive tract of detritivores or predators (Matthews and Matthews 1980). Some authors have suggested the possibility of intermediate hosts in the life cycle of some Microsporidia (Weissenberg 1911; Bekhti et al. 1985; Amigó et al. 1993). Both *S. maximus* and *L. budegassa* are bottom-living fishes; they share sandy and muddy bottoms of the Mediterranean sea (Sostoa 1990). Their feeding habits are also similar, both feeding on benthonic prey (Corbera et al. 1996). This observation could be related to the pathway of transmission of the parasite, although this has yet to be established. One possible explanation could be that these anglers could have eaten infected turbots; it is well known that *L. budegassa* is a very voracious species (Corbera et al. 1996), and the ingestion of flatfish (Pleuronectiformes) by *L. budegassa* has also been reported (Pereda et al. 1984). The presence of *T. brevifilum* in *L. budegassa*, although rare, could be taken as an example of the importance that wild species might have as a reservoir for parasites.

The exceptional resistance of microsporidian spores (Amigó et al. 1996b), together with the use of fish residues (such as angler), either fresh, frozen or dried, for the production of food for fish farms, should be considered as a possible pathway of dispersion of *T. brevifilum*. Recently, the farming of *S. maximus* in captivity has been considered off the Catalan coast (Northeast Spain; Castelló Orvay 1993). The presence of *T. brevifilum* on this coast should be considered as a potential threat to the culture of this species.

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