

# *Kudoa unicastula* n. sp. (Myxosporea: Kudoidea) a parasite of the Mediterranean mullets *Liza ramada* and *L. aurata* (Teleostei: Mugilidae)

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**Abstract** A new multivalvulid myxozoan parasite, *Kudoa unicastula* n. sp., is described from the intestinal mesentery, intestine and pyloric caeca of the thin-lipped grey mullet *Liza ramada* (Risso 1826) and the golden grey mullet *L. aurata* (Risso, 1810) from the Mediterranean coastal waters of Spain. It is characterized by the presence of elongated, rice corn-like white cysts of 0.47–0.56 × 0.18–0.38 mm, filled with tetracapsulate, slightly asymmetric spores, rectangular in apical view and tear-shaped in lateral view with four polar capsules of considerably different size and slightly unequal spore valves with rounded edges,

overlapping each other on the apex of the spore. One large polar capsule includes a polar filament coiled in two to three turns, and the other three polar capsules, which are very small, possess only a rudimentary filament. Both light and electron microscopy data showed that this species differs from all previously described *Kudoa* spp. with unequal polar capsules. The molecular analysis based on 18S and 28S ribosomal deoxyribonucleic acid DNA sequence data of *K. unicastula* n. sp. indicates a close relationship and thus phylogenetic clustering together with *K. trifolia*, a myxozoan from the same host and the same geographical location.

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## Introduction

Currently, 84 species, including 25 unnamed, belong to the genus *Kudoa* Meglitch 1947. Most species of the genus are histozoic parasites of more than 180 species of marine and estuarine fishes. Thirty-seven identified and 19 unnamed species infect the muscle, while the others occur in different internal organs. Five species have been found in the mesentery associated with the intestine and in the pyloric caeca of fish. Some representatives of the genus *Kudoa* cause important economical losses because of the reduction in the market value of products from infected fish (e.g. *K. azoni* Aseeva 2004, *K. megacapsula* Yokoyama and Itoh 2005, *K. thyrstites* (Gilchrist 1924), *K. histolytica* (Perard 1928), *K. musculoliquefaciens* (Matsumoto 1954), *K. amamiensis* Egusa and Nakajima 1980, *K. miniauriculata* Whitaker, Kent and Sakanari 1996, *K. rosenbuschi* (Gelormini 1943); Whitaker et al. 1994; Aseeva 2004; Moran et al. 1999; Naidenova Gaevskaya 1991) but also because of superficial ulceration of the fish skin (e.g. *K. chupeidae* (Hahn 1917), *K. mirabilis* Naidenova and Gaevskaya 1991).

Myxozoans with apparently unicapsulate spores were found during a parasitological study of mullets from the estuary of the River Ebro and from littoral parts off the Spanish Mediterranean coast, in May 2004 and June 2005. The ultrastructural analysis exposed three reduced polar capsules, and together with molecular data, the species presented itself as a member of the genus *Kudoa*. In the present study, the spore morphology, ultrastructural features and molecular identity of this new myxozoan species are presented.

## Materials and methods

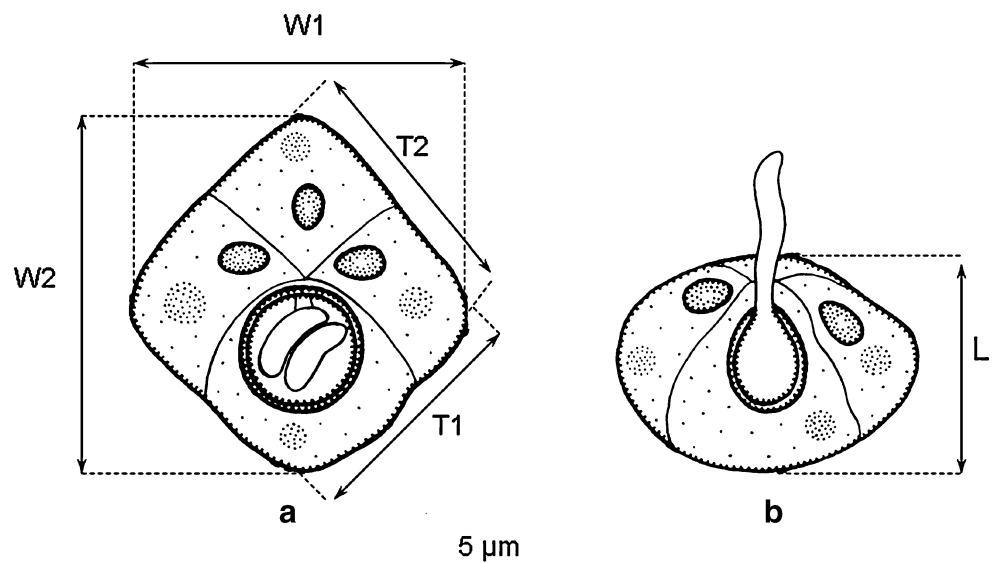
Mullets were purchased at local fish markets from three localities in the Spanish Mediterranean during May/June 2004 and 2005 namely, from Santa Pola Bay, Santa Pola Salt Marshes and the River Ebro Delta. A total of 350 specimens, belonging to five species of Mediterranean mullets were examined: flathead mullet *Mugil cephalus* Linnaeus 1758 (179 specimens), golden grey mullet *Liza aurata* (Risso 1810) (121 specimens), thin-lip mullet *L. ramada* (Risso 1826) (29 specimens), leaping mullet *L. saliens* (Risso 1810) (ten specimens) and thick-lip grey mullet *Chelon labrosus* (Risso 1827) (ten specimens). Fish were transported to the laboratory on ice and dissected on arrival according to a standardised protocol specifically developed for mullets (Kostadinova et al. 2004). Morphological and molecular data of the myxozoan described in this study were obtained from fresh fish, whereas fish used for prevalence data were frozen at  $-20^{\circ}\text{C}$  and thawed on the day of examination. Skin, gills, brain, digestive tract, gall and swim bladder, liver, kidney, heart, spleen, muscle and eyes were examined for myxosporean infections. Fresh preparations of spores on slides were embedded in gelatin–

glycerin (1:1). Spores were photographed and measured on digital images. Descriptions of the spores were based on the guidelines of Lom and Arthur (1989), Lom and Dykova (1992) and Schulman et al. (1997). According of Lom and Dykova (1992), the measurements of *Kudoa* spp. include width, length and thickness of the spore. Some additional measurements of spore width and length were taken because of existing asymmetry (see Fig. 1 for definition of new spore measurements).

For ultrastructural analyses, infected tissues were fixed in a 2.5% (v/v) glutaraldehyde in 0.1 M sodium cacodylate buffer (pH 7.4) for several days at  $4^{\circ}\text{C}$ . After washing twice with 0.1 M sodium cacodylate buffer and post-fixation in 2.0% (v/v) osmium tetroxide in cacodylate buffer for 1 h at  $4^{\circ}\text{C}$ , the pieces were dehydrated and embedded in Epon–Araldite solution using a standard procedure (Vavra and Maddox 1976). Blocks of embedded tissues were sectioned with an LKB III ultra-microtome. Semi-thin sections were stained with methylene blue. Ultra-thin sections were mounted on copper grids, double stained with uranyl acetate and lead citrate and examined in a JEM 100B electron microscope operated at 80 kV.

For molecular analysis, deoxyribonucleic acid (DNA) was extracted from three individual rice corn-sized plasmodia from three individuals of *L. ramada*. Isolated plasmodia were transferred to TNES–urea, and DNA was extracted using a phenol–chloroform protocol as previously described (Holzer et al. 2004). 18S ribosomal DNA (rDNA) was amplified using universal eukaryotic 18S primers (ERIB1: 5'-ACCTGGTTGATCCTGCCAG-3' and ERIB10: 5'-CTTCCGCAGTTCACCTACGG-3', Barta et al. 1997). Sequencing reactions were carried out as in Holzer et al. 2006. The polymerase chain reaction (PCR) products obtained were purified for sequencing using the High-Pure PCR Product Purification Kit (Roche Diagnostics, Germany).

**Fig. 1** Diagram of mature spore of *K. unicapsula* n. sp. in apical (a) and lateral (b) view and method of measurement. *W1*, *W2* width of the spore, *T1*, *T2* thickness of the spore, *L* length of the spore



Primers ERIB1 and ERIB10 (see above) as well as LIN3 (5'-GCGGTAATTCCAGCTCCA-3', Lin et al. 1999) and LIN10 (5'-CACTCCACGAACTAAGAA-3', Lin et al. 1999) were used for cycle sequencing of the 18S fragments in a 48 capillary ABI 3730 sequencer (Applied Biosystems) using the BIG Dye Terminator v3.1 Ready Sequencing Kit (Applied Biosystems). Because of the close molecular similarity of the 18S rDNA sequences of the present myxozoan with that of *K. trifolia*, additional 28S rDNA sequences were obtained for both species, using primers Kt28S1F (5'-CAAGACTACC TGCTGAAC-3', Whipps et al. 2004) and 28S1R (5'-GTGTTTCAAGACGGGTCG-3', Whipps et al. 2004). Sequences of *K. trifolia* were obtained from two samples used for sequencing of the 18S rDNA (Holzer et al. 2006). Sequence alignment with other *Kudoa* spp. was conducted using Clustal X (Thompson et al. 1997). Tree inference was carried out by a likelihood-based Bayesian tree sampling procedure using MrBayes v 3.0 (Ronquist and Huelsenbeck 2003) with parameters corresponding to the general time-reversible model GTR+I+ $\Gamma$  (Tamura and Nei 1993). The MCMC was allowed to run for 1,000,000 generations sampling every 100th tree. The first 24,000 generations were later discarded as the burn-in period.

## Results

The following are the characteristics of *Kudoa unicapsula* sp. n.:

Type hosts: Thin-lip mullet *Liza ramada* (Risso 1826) and golden grey mullet *L. aurata* (Risso, 1810)

Prevalence: 40% (10/25) in *L. ramada* and 15% (18/121) in *L. aurata*

Type localities: Mediterranean; Santa Pola Bay (38°11'N, 0°34'W) and Ebro Delta (40°42'N, 0°43'W), Spain

Site of infection: Intestinal mesentery, intestine, and pyloric caeca

Pathogenicity: Parasite plasmodia were sometimes encapsulated by fibrous connective tissue (Fig. 2b,c) but no other host tissue reactions were observed. Infection caused no clinical signs in any of the fish species

Etymology: Species name refers to the presence of only one large, easily visible polar capsule and three reduced polar capsules, which are difficult to be discriminated by light microscopy

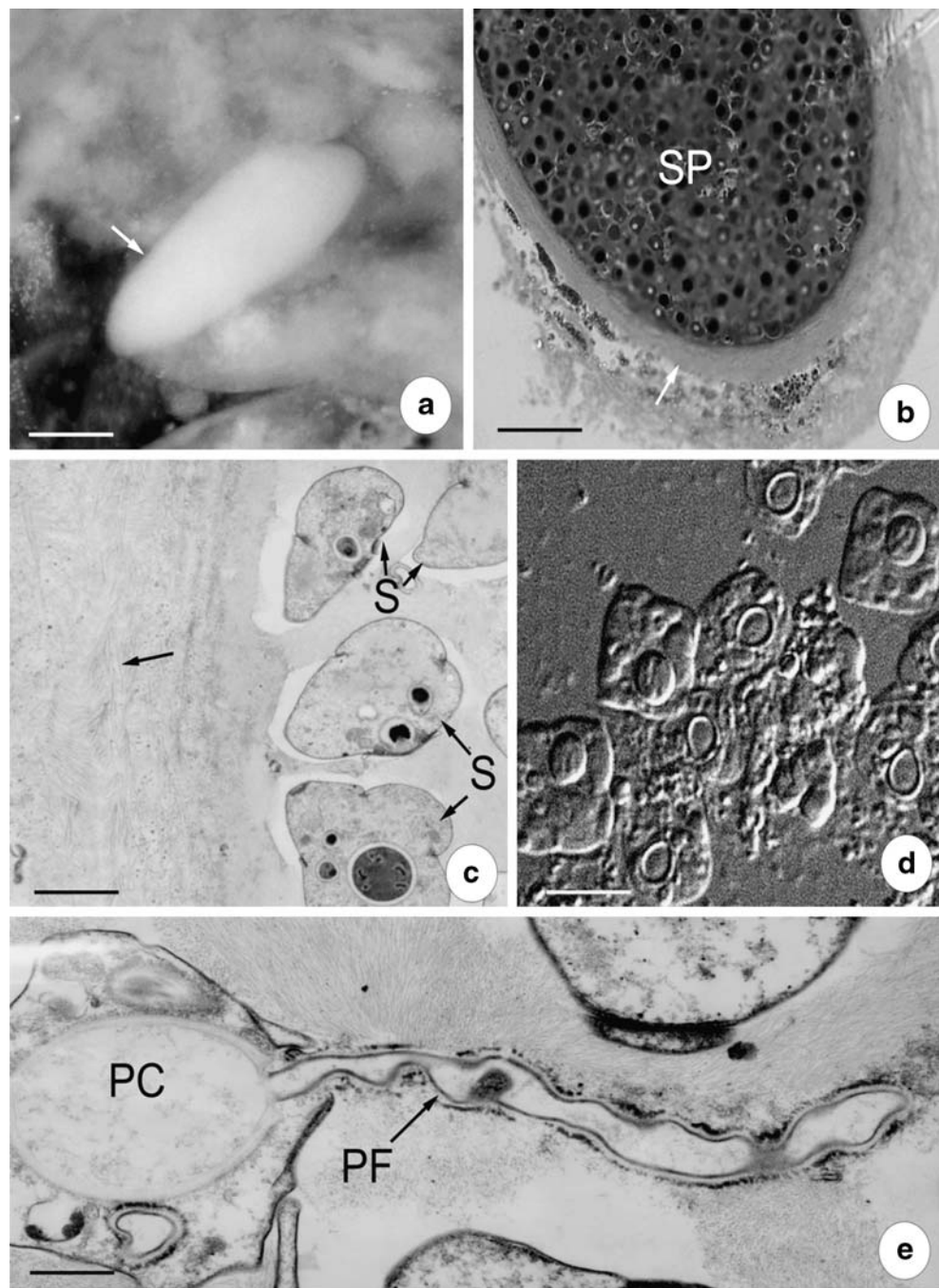
Material deposited: Syntypes of gelatin–glycerin-embedded spores on slides 1 MRE 1, 11; 2 MRE 11, 23; 2 MRS 35, 45, 48, 50, 59; 3 MRS 6; 2 MAE 38 were deposited in Department of Ecological Parasitology, Institute of Biology of the Southern Seas, Sevastopol, Ukraine, and in the Microbiology Slide Collection of the Natural History Museum, London, UK

Light microscopy data (Figs. 1, 2a,b,d; Table 1): Plasmodia polysporous, elongated, rice corn-like in shape, 0.47–0.56 × 0.18–0.38 mm ( $n=20$ ) in size (Fig. 2a). Spores asymmetrical, rectangular in apical view and drop-shaped in lateral view with slightly flattened apical and rounded ventral pole. Polar capsules ovoid, distinctly different in size (Fig. 2d): One large polar capsule with polar filament in two to three coils and three degenerate polar capsules, inconspicuous by light microscopical observation. Four slightly unequal shell valves enclosing the spore contents. Valve containing large capsule slightly bigger than the others and rounded. Other three shell valves smaller, with straighter edges. Spore measurements are presented in Table 1.

Transmission electron microscopy (Figs. 2c,e, 3): Parasite plasmodium encapsulated by collagenous border of 0.04 mm width containing numerous, sometimes interconnected microfibrils (Fig. 2c). Shell valves of spore 70 nm thick, adhering along sutural lines (Fig. 3a). Valve edges round. Thickened valve flanges of 150 nm in width overlapping on apical pole of the spore (Fig. 3d,f). Overlapping parts of shell valves contain regular patches of electron-dense material (Fig. 3d). Only one large polar capsule fully developed in mature spore (Fig. 3a,d,g). Remaining three capsules degenerated with weakly developed polar filaments (Fig. 3a,b,e). Large polar capsule with 230-nm-wide wall consisting of two layers, i.e. an internal, electron-lucent layer, 170 nm wide and an external, finely granular electron-dense cover (width?). Both layers continue into polar filament wall (Fig. 3d,g). Polar capsule opening filled with finely granular material of the same structure as outer layer of capsule wall (Fig. 3d). Capsular primordium of immature spore composed of finely granular matrix core and coarse, electron-dense cortex of future polar filament (Fig. 3f). Polar filament appears as a flattened, S-like tube in cross-sections, 200 nm thick and 310 nm wide, with an outer, electron-transparent layer, 70 nm wide and an inner, electron-dense layer, 50 nm wide. Discharged polar filament electron dense (Fig. 2e). Degenerate polar capsules with low number of electron-dense units (Fig. 3c). Sporoplasm consists of two uninucleate cells, a primary cell enveloping a secondary cell (Fig. 3c). Membrane-bound vacuole measures 700 nm in diameter and harbours one of sporoplasm nuclei (Fig. 3f).

Molecular data (Fig. 4): 18S rDNA sequencing of *K. unicapsula* n. sp. produced a 1,505-bp sequence (GenBank accession number AM490334). Alignment with 18S rDNA sequences of *Kudoa* spp. identified *K. unicapsula* n. sp. as a member of the genus and showed close sequence similarity between *K. unicapsula* n. sp.

**Fig. 2** *K. unicapsula* n. sp. light and ultrastructural images of plasmodial (a, b, c) and spore (d, e) morphology. **a** Elongated rice corn-like plasmodia (arrow) form the pyloric caeca of *Liza aurata*. **b** Semi-thin section of spore forming plasmodium stained with methylene blue. The plasmodium is surrounded by host connective tissue (arrow). **c** Ultra-thin section of part of the same plasmodium as in **b**. Collagen microfibrils (arrowed) and mature spores (S) are visible. **d** Interference-contrast image of fresh spores of *K. unicapsula* n. sp. **e** Ultrathin section of the spore with polar filament (PF) extruded from large polar capsule (PC). Scale bars: **a**=0.2 mm, **b**=0.1 mm, **c**=2.0  $\mu$ m, **d**=4.8  $\mu$ m, **e**=1.0  $\mu$ m



and *K. trifolia* (99.5%), a myxozoan from the same geographical area and the same mugilid hosts. The comparison of 28S rDNA sequences of *K. unicapsula* n. sp. (728 bp, AM490335) and *K. trifolia* (795 bp, AM490336) showed only slightly lower (89.8%) sequence identity between the two species. As a result of the high sequence similarity, the two species clustered together with 1.00 clade posterior probability in both the 18S (not shown) and the 28S (Fig. 4) rDNA phylogenetic analysis of *Kudoa*. This signifies that all the trees

sampled during the analysis contained the *K. unicapsula* n. sp./*K. trifolia* branch.

**Taxonomic remarks:** The myxozoan parasite described can be placed in *Kudoa* Meglitsch, 1947 based on the shape and structure of the spore but also because of its molecular similarity to other representatives of the genus. To date, nine species of *Kudoa* have been reported to parasitize mullets. Five of them, *K. cascasia*, *K. chilkaensis*, *K. ciliatae*, *K. diana* and *K. iwatai*, were recorded from the mesentery surrounding

**Table 1** Spore measurements

| Spore ranging names             | Fresh spores ( $n=20$ ) |         | Glycerin–gelatine preparations ( $n=60$ ) |         |
|---------------------------------|-------------------------|---------|---|---------|
|                                 | Mean $\pm$ SD           | Range   | Mean $\pm$ SD                             | Range   |
| Width max (W2)                  | 8.0 $\pm$ 0.33          | 7.2–8.4 | 6.5 $\pm$ 0.41                            | 5.5–7.5 |
| Width min (W1)                  | 7.7 $\pm$ 0.53          | 6.6–8.3 | 6.0 $\pm$ 0.62                            | 4.4–7.2 |
| Thickness max (T2)              | 6.1 $\pm$ 0.39          | 5.3–6.9 | No data                                   | No data |
| Thickness min (T1)              | 5.5 $\pm$ 0.43          | 4.7–6.3 | No data                                   | No data |
| Length (L)                      | 5.0 $\pm$ 0.24          | 4.7–5.5 | 5.7 $\pm$ 0.43                            | 5.1–6.2 |
| Length of large polar capsule   | 3.1 $\pm$ 0.15          | 2.8–3.4 | 2.5 $\pm$ 0.24                            | 1.8–2.9 |
| Length of middle polar capsules | 1.4 $\pm$ 0.08          | 1.3–1.5 | No data                                   | No data |
| Length of small polar capsule   | 0.8 $\pm$ 0.05          | 0.8–0.9 | No data                                   | No data |
| Width of large polar capsule    | 2.7 $\pm$ 0.11          | 2.5–2.9 | 2.0 $\pm$ 0.16                            | 1.5–2.3 |
| Width of middle polar capsules  | 0.8 $\pm$ 0.06          | 0.7–0.9 | No data                                   | No data |
| Width of small polar capsule    | 0.7 $\pm$ 0.05          | 0.7–0.8 | No data                                   | No data |
| Polar filament length           | 7.1 $\pm$ 0.71          | 5.9–8.0 | 7 $\pm$ 0.62                              | 5.9–8.8 |
| Polar filament width            | 0.4 $\pm$ 0.16          | 0.3–0.6 | 0.5 $\pm$ 0.16                            | 0.2–0.6 |

the intestine and from the pyloric caeca of mullets from commercial fish of the Indian and the Pacific Oceans (Sarkar and Chaudhury 1996; Tripathi 1951; Lom et al. 1992; Dykova et al. 2002; Egusa and Shiomitsu, 1983). The spore morphology of *K. unicapsula* n. sp. is distinct from all these species because of the presence of one large and three very small, degenerate polar capsules. Unequal polar capsules have been described from eight *Kudoa* species (Table 2). Of these, *K. unicapsula* n. sp. most closely resembles *Kudoa* sp. Bunton and Poynton 1991 infecting *Morone americana* (Gmelin) of North American coastal waters (Bunton and Poynton 1991). Both species were recorded in euryhaline waters but differ by hosts and tissue location. However, differences in shape and/or morphometry exist between *K. unicapsula* n. sp. and all species summarized in Table 2, so that the unique morphology and molecular characteristics classify it as a new species.

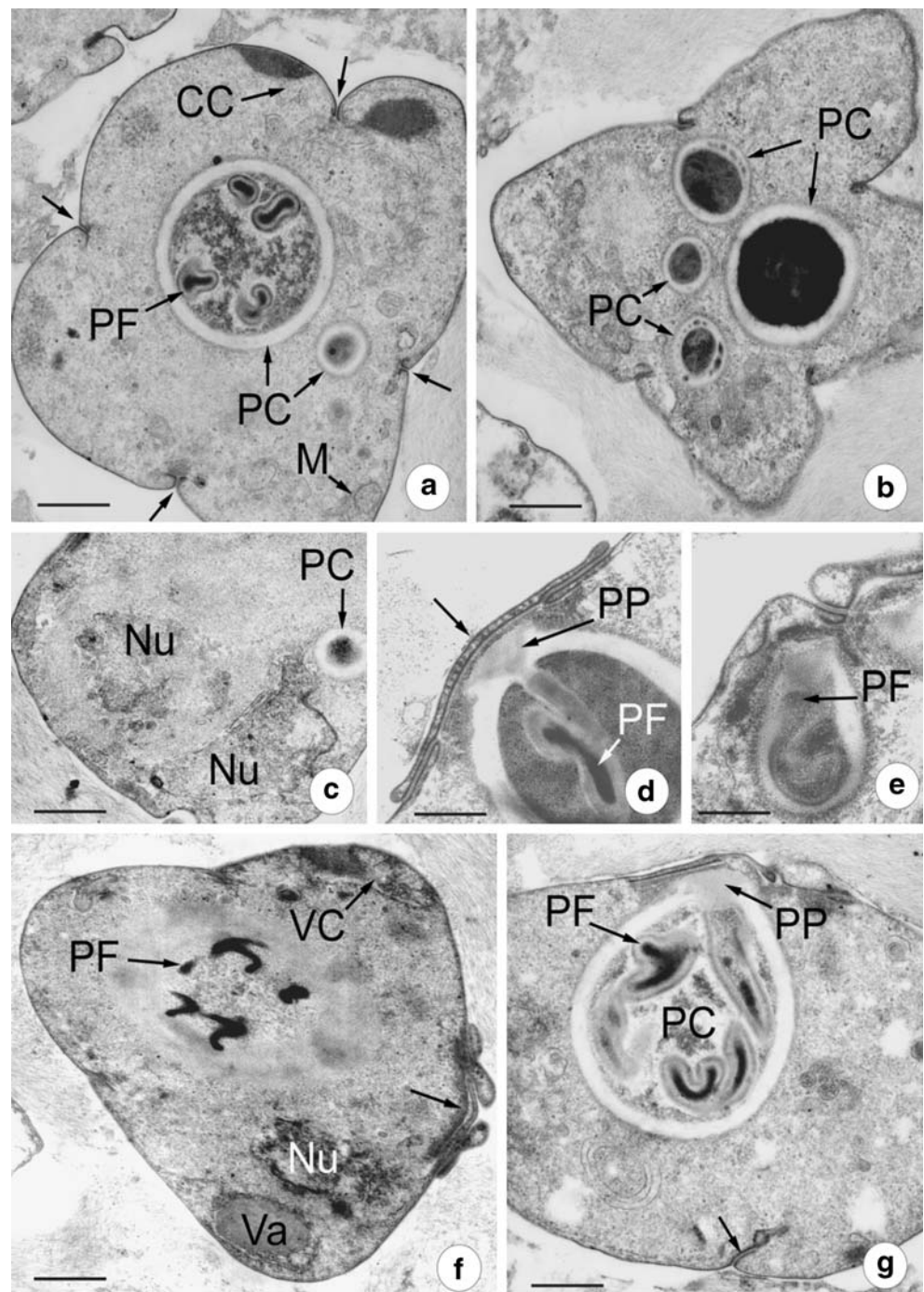
## Discussion

Out of five examined mullet species from the Spanish Mediterranean, only two, *L. ramada* and *L. aurata*, were infected by *K. unicapsula* n. sp. indicating certain host specificity. *K. unicapsula* n. sp. was found in wild population of grey mullets from the Santa Pola Bay and the Ebro Delta but not in the Santa Pola Salt Marshes. Today, these marshes are not used for salt production any more but are filled with freshwater from the adjacent river and used for freshwater aquaculture. This might be indicative for the presence of the alternate host in marine and brackish water but not in freshwater. This is in

accordance with the fact that *Kudoa* is a generally marine genus, which might be dependent on generally marine alternate host species.

Despite the three reduced polar capsules, *K. unicapsula* n. sp. shows a morphology that is typical for *Kudoa*, with four polar capsules and subquadrate spores. However, its closest relative on a molecular basis (18S and 28S rDNA), i.e. *K. trifolia*, shows a considerably different morphology because of the extensive enlargement of a valve cell. At the same time, these two species show a large amount of molecular identity in two ribosomal gene regions, with only 0.5 (18S) and 10.2% (28S) sequence divergence. A similar amount of divergence (0.2–1%) can be found between the 18S rDNA sequences of different geographic isolates of *K. thyrssites*; however, *K. thyrssites* intraspecific divergence is lower (3–9.6%) than the one between *K. unicapsula* n.sp. and *K. trifolia* (10.2%) when the 28S rDNA region is compared (Whipps and Kent 2006). The sequence data of *K. thyrssites* also shows that 28S rDNA divergence between geographical isolates from the same oceanic region (Eastern Pacific, Atlantic, Australia and Japan) is below 1% for 28S, but *K. unicapsula* n. sp. and *K. trifolia* occur in a very small enclosed habitat, i.e. the Mediterranean with much higher sequence divergence. Interspecific variance between the members of *Kudoa* is 1–28% for 18S rDNA and 9.4–33% for 28S rDNA. This shows that on the basis of the 18S rDNA, *K. unicapsula* n. sp. and *K. trifolia* could be conspecific but not when 28S rDNA sequences are compared. However, it has been indicated several times that the 18S rDNA sequence is of limited use in clarifying the systematics of closely related myxozoans (e.g. Kallert et al. 2006; Fiala 2006). Another feature, i.e. site preference, strongly indicates that *K. unicapsula* n. sp. and *K. trifolia* are not conspecific: *K. unicapsula* n. sp. occurs exclusively in the pyloric caeca, intestine and intestinal

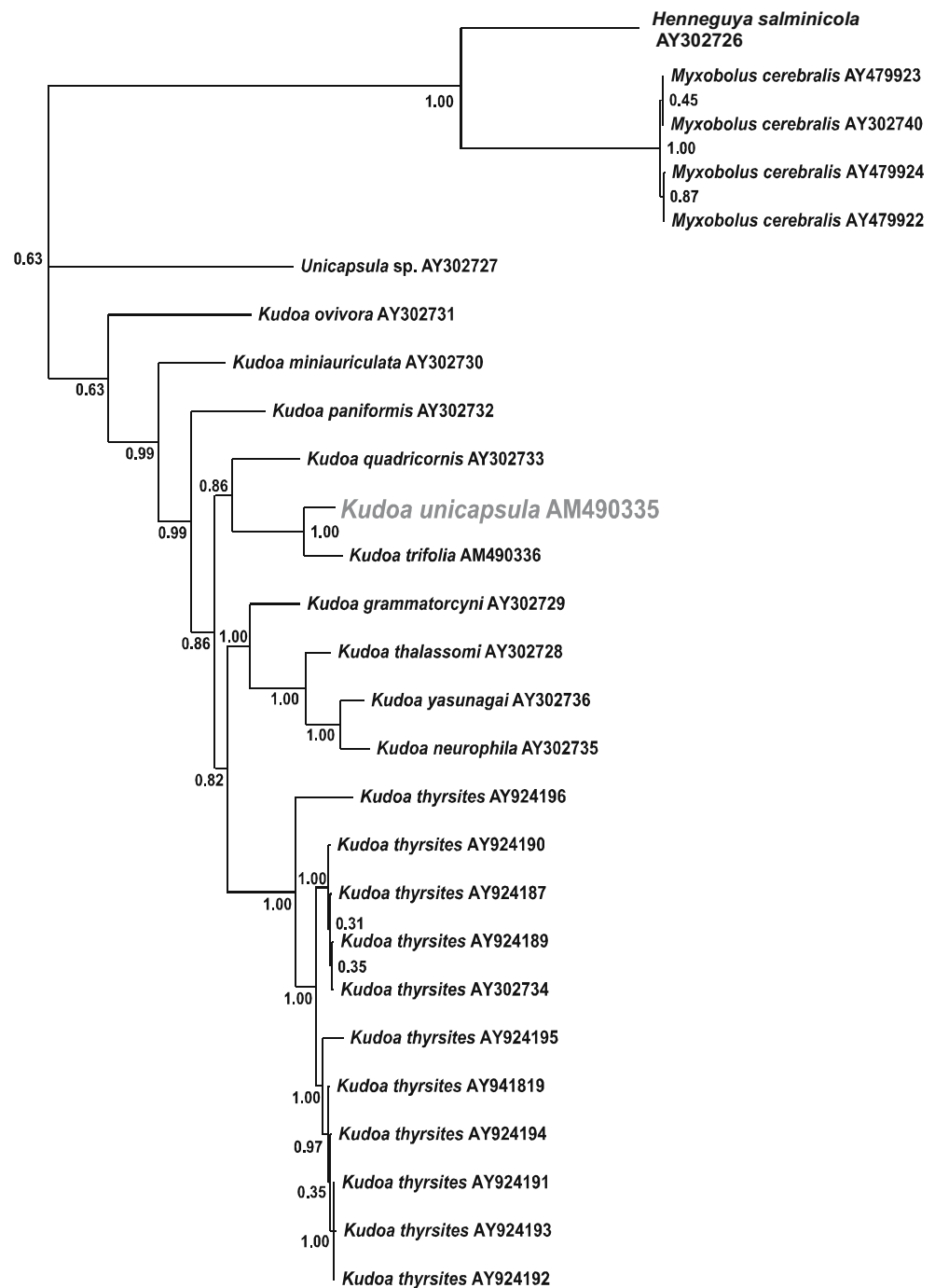
**Fig. 3** Ultrastructural features of mature spores of *K. unicapsula* n. sp. **a** Transverse section through basal part of the immature spore showing two polar capsules (PC), mitochondrion (M), valvogenic cells (VC) and suture lines (arrows). Big polar capsule contains two coils of polar filament (PF). **b** Cross-section of mature spore showing four unequally sized polar capsules (PC). **c** Spore with two unequal nuclei (Nu) and small polar capsule (PC). **d** Longitudinally sectioned large polar capsule with polar filament arranged in longitudinal coils. The polar capsular opening (PP) is filled by finely granular material of the same structure as outer layer of capsule wall. The polar capsule entrance is covered by the shell valve flanges containing patches of electron dense material. **e** Longitudinal section of small polar capsule containing weakly developed polar filament (PF). **f** Diagonally sectioned immature spore. Four shell valves are overlap at the spore apex (arrowed). Valvogenic cell (VC) and capsulogenic cell with developing polar filament (PF) and nucleus (Nu) are shown. Membrane-bounded vacuole (Va) is filled by amorphous substance. **g** Longitudinal section of spore. Large polar capsule (PC) contains fully developed polar filament. End of polar filament (PP) is located near junction of shell valves. *Scale bars:* **a**=1.1 mm, **b**=1.0 mm; **c**=1.2 mm, **d**=0.8 μm, **e**=0.5 μm, **f**=1.1 μm, **g**=0.8 μm



mesentery, whereas *K. trifolia* can be found in various sites in and along the digestive tract and in other internal organs but in contrast to *K. unicapsula* n. sp. is always closely associated with connective tissue (Holzer et al. 2006). Site of infection assists in the identification of myxozoan species, as tissue tropism and cyst location in some cases have been shown to be more important than morphology, geographical distribution or host specificity (Eszterbauer 2004; Fiala 2006). Spore morphology might also confirm the establishment of the new species *K. unicapsula* n. sp. to

some degree. Morphological characters of the spores can show some variability within the same species as the number of polar capsules and valve cells vary in *Kudoa* (Egusa 1986; Whipps et al. 2003) or the length of floating appendages in actinosporeans (Hallett et al. 2002). Thus, spore morphology is the most limited of all proofs for establishing a new species. However, in the present case, the morphological differences between *K. unicapsula* n. sp. and *K. trifolia* do not represent a simple multiplication or elongation of a spore part but would require a combination

**Fig. 4** Phylogenetic tree based on Bayesian inference (GTR+I+ $\Gamma$ ) of 28S rDNA data of *Kudoa* spp. showing position of *K. unicapsula* n. sp. *Henneguya salminicola* and *Myxobolus cerebralis* are out-group species. The numbers at the nodes show the clade posterior probability, which is the proportion of the sampled trees containing that branch



of structural changes, i.e. shrinking of a strongly enlarged polar capsule and considerable expansion of a valve cell with allocation of the sporoplasm within it. Such drastic changes have not been observed between spores of the same species to date. As both species inhabit the same mugilid hosts in the same geographical area (Spanish Mediterranean), it is possible that speciation of these two representatives of *Kudoa* has been going on for a relatively short time, resulting in small genetic differences and morphological speciation of *K. unicapsula* sp. n., and *K. trifolia* might be more pronounced than the one observed

on a molecular basis. Summarizing, the combination of 28S rDNA data, host tissue location and morphology lead us to conclude that *K. unicapsula* n. sp. should be considered a new valid species.

To observe and interpret small molecular differences between species or geographical isolates, genes other than the 18S rDNA, which is commonly used for myxozoan, should be tested for their suitability for phylogenetic studies. Whipps and Kent (2006) made a successful approach involving the more variable ribosomal gene regions (28S, ITS1 and ITS2) as well as sequences of heat

**Table 2** Characteristics of different *Kudoa* species

| Species  | Spore ( $\mu\text{m}$ )   |           |          |           | Polar capsule ( $\mu\text{m}$ )  |           |           | Host, sites   |
|--|---|-----------|----------|-----------|--|-----------|-----------|---|
|  | Shape   | Width     | Length   | Thickness | Shape  | Width     | Length    |   |
| <i>K. minithyrsites</i> Whipps et al. 2001       | Stellate in apical view   | 7.8–9.9   | 4.3–5.4  | 5.8–7.5   | Convergent, pyriform, one larger than other three                                    | 1.3–2.3   | 3.2–3.8   | <i>Pempheris ypsilychnus</i> , somatic muscle                               |
| <i>K. valamugili</i> Kalavati et al. 1993        | Spore quadrate in apical view. Valves unequal with rounded edges                                    | 5–5.4     | 5.2–6.6  | 4.3–5.6   | Pyriform, one large, three small equally sized                                       | 1.6–2     | 3–3.8     | <i>Valamugil cunnesius</i> , intestinal muscle                              |
| <i>K. mirabilis</i> Naidenova and Gaevskaya 1991 | Stellate in apical view, valves unequal   | 10.3–10.4 | 6.6–8.8  | 8.1–12    | Ovoid, one large, three small equally sized  | 4.7–5.2   | 5.9–7.7   | <i>Trichiurus lepturus</i> , muscle   |
| <i>K. megacapsula</i> Yokoyama and Itoh 2005     | Stellate to cruciform in apical view, with protections  | 11–12.9   | 9.3–15.4 | 30.3–44.7 | Pyriform, one distinctly large, two small equally sized, one vestigial               | 4.9–6.8   | 11.7–14.2 | <i>Sphyræna pinguis</i> , muscle  |
| <i>K. stellula</i> Yurakhno 1991                 | Quadrate in apical view with sharp edges  | 5–6.9     | No data  | 3.7–4.7   | Pyriform, one large, two middle, one small sized                                     | 1.3–1.5   | 2–2.2     | <i>Atherina hepsetus</i> , kidney   |
| <i>K. tachysuræ</i> Sarkar et Mazumder 1983      | Quadrate in apical view with rounded valves   | 7–9       | 4.5–6    | 5–6.5     | Pyriform, one large, three small equally sized                                       | 2–3       | 3–4       | <i>Tachysurus tenuispinis</i> , gall bladder                                |
| <i>Kudoa thyrsites</i> (Gilchrist 1924)          | Stellate in apical view. Valves unequal with sharply elongated edges                                | 5.5–7     | 5–8      | 11–12.5   | Pyriform, one large, two middle, one small sized                                     | 1–1.5     | 1–1.5     | <i>Thyrsites atun</i> , and 34 others                                       |
| <i>Kudoa</i> sp. Bunton and Poynton 1991         | Subquadrangular in apical view, rounded and pyramidal in lateral view                               | 5.5–8     | 4–6      | 5–6.5     | Pyriform, one distinctly large, three small equally sized                            | Up to 2.9 | 2.7–3.5   | fish species (Moran et al. 1999), somatic muscle                            |
| <i>K. unicasula</i> sp. N.                       | Rectangular in apical view, drop-shaped in lateral view. Valves slightly unequal with rounded edges | 7.2–8.4   | 4.7–5.5  | 5.3–6.9   | Ovoid, one capsule distinctly larger than other three, degenerative, unequally sized | 2–3       | 2.8–3.4   | <i>Liza aurata</i> , <i>L. ramada</i> , intestinal mesentery, pyloric caeca |
|  |   | 6.6–8.3   | 4.7–6.3  | 4.7–6.3   |  | 1–1.3     | 2–3       |   |



shock protein 70 for a phylogeographic study of *K. thyrssites* isolates. In the current study, the 28S rDNA sequences published by these authors were analysed phylogenetically together with the sequences obtained for *K. unicapsula* n. sp. and *K. trifolia*. The tree topology produced by the analysis of *Kudoa* spp. 28S rDNA sequences differs from the one produced by 18S rDNA sequences (see, e.g. Holzer et al. 2006; Diamant et al. 2005), with notably higher confidence values obtained in the 28S rDNA analysis, which can partially be ascribed to the higher sequence variability between different species of *Kudoa*. This suggests that this gene region could be useful for the analysis of phylogenetic relationships within closely related species of Myxozoa.

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