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Ultrastructure and phylogeny of *Glugea arabica* n. sp. (Microsporidia), infecting the marine fish *Epinephelus polyphekadion* from the Red Sea

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Abstract

A new microsporidian species, *Glugea arabica* n. sp., is reported infecting the intestinal wall of the marine teleost *Epinephelus polyphekadion* (=*microdon*) collected from the Red Sea coast off Saudi Arabia, and described on the basis of microscopic and molecular procedures. Spherical blackish xenomas formed parasitophorous vacuoles completely packed with several parasitic developmental stages, including spores. The nuclei were monokaryotic in all developmental stages. Spores were ellipsoidal to pyriform and measured 6.3 ± 0.3 (5.9–6.6) μm in length and 3.3 ± 0.4 (2.9–3.7) μm in width. A lamellar polaroplast surrounded the uncoiled portion of the polar filament, which extended into the spore's posterior pole and formed 27–29 coils organized in three or four rows. The posterior vacuole, located at the spore's posterior pole, appeared surrounded by the polar filament coils and displayed an irregular matrix composed of light material, in which was located the posterosome. Molecular analysis of the rRNA genes, including the ITS region, was performed using maximum parsimony, neighbor-joining and maximum likelihood methodologies. The ultrastructural features observed, in combination with the molecular data analysed, suggests the parasite to be a new species of the genus *Glugea*.

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Keywords: Fish parasite; *Glugea arabica* n. sp.; Microsporidians; Phylogeny; Red Sea; Ultrastructure

Introduction

Members of the phylum Microsporidia Balbiani, 1882 are obligate intracellular parasites characterized by the absence of cellular components typical of eukaryotes, such as mitochondria, Golgi apparatus and flagella (Corradi and Selman 2013). These parasites infect several groups of invertebrate hosts, namely arthropods, and all vertebrate

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classes (Canning and Lom 1986; Larsson 1999; Lom and Dyková 1992), including humans (Vossbrinck and Debrunner-Vossbrinck 2005; Weiss and Vossbrinck 1998).

The most common vertebrate hosts of microsporidia are fish, accounting for infections caused by over 100 species distributed among 18 genera (Baquero et al. 2005; Casal et al. 2008; Diamant et al. 2010, 2014; Lom 2008). Among these genera, the genus *Glugea* Thélohan, 1891 is one of the largest, containing species that infect a wide range of marine and freshwater fish (Lom 2002; Vagelli et al. 2005).

Members of the genus *Epinephelus* of the family Serranidae are large tropical and subtropical sea fish, commonly known as groupers. About 25 species of this genus are known to inhabit the water of the Red Sea. The camouflage grouper *Epinephelus polyphekadion* is usually captured along the nearshore coral reef area of the southern Red Sea coast off Saudi Arabia (James et al. 1997), and presents a high marketable potential. Recently, some microsporidian species were described in fish from the Red Sea, including from serranid fish hosts. *Glugea nagelia* is one such species, which has been described infecting the intestinal wall of the yellow hind, *Cephalopholis hemistictos* caught in the Red Sea coast off Saudi Arabia (Abdel-Baki et al. 2015).

From the Red Sea coast off Egypt, meanwhile, several *Pleistophora* spp. have been referenced, inclusively from fish hosts of the genus *Epinephelus*, but these have mostly been reported without molecular data (Abdel-Ghaffar et al. 2009, 2011, 2012; Morsy et al. 2012). Only *P. pagri* and *P. aegyptiaca* combine an ultrastructural and molecular description, the first having been described from the epithelial lining of the peritoneum and the intestinal epithelium of the common sea bream *Pagrus pagrus* (Sparidae) (Morsy et al. 2012), while the second forms xenomas in the peritoneal cavity of *Saurida tumbil* (Synodontidae) (Abdel-Ghaffar et al. 2012; Morsy et al. 2012). Also from this geographical area, *Microsporidium aurata* has been described forming xenomas that adhered to the muscle, connective tissues and intestinal epithelium within the peritoneal cavity of the gilthead seabream *Sparus aurata* (Sparidae); however this species awaits further taxonomic classification at the genus level (Morsy et al. 2013). A gonadotropic microsporidian parasite, *Obruspora papernae* n. gen. and n. sp. was recently described from the blotchfin dragonet *Callionymus filamentosus* (Callionymidae) in the Mediterranean Sea. This fish species is common in Red Sea waters and enters the Mediterranean Sea through the Suez Canal (Diamant et al. 2014).

Most descriptions of microsporidian parasites are based on their cellular structure, life cycle, and host specificity (Corradi and Keeling 2009), however, molecular analysis of the rRNA genes has become a requisite for the comprehension of microsporidian taxonomy and phylogeny (Vossbrinck and Debrunner-Vossbrinck 2005; Weiss and Vossbrinck 1999). In the case of the genus *Glugea*, GenBank provides rRNA sequences for only nine species, and this paucity molecular data hampers the phylogenetic study of this genus. Acknowledging the necessity of using combined microscopic and

molecular procedures, the present study describes a new *Glugea* species that was found infecting the camouflage grouper in the Red Sea coast off Saudi Arabia.

Material and Methods

Fish sampling

Twenty-four specimens of the camouflage grouper *Epinephelus polyphekadion* (Teleostei, Serranidae), with an average length of ~60 cm, were collected from the Red Sea coast near the city Jeddah (21°31' N, 39°13' E), Saudi Arabia. Upon necropsy, parasitic infection was detected in the form of spherical blackish xenomas that adhered to the intestinal wall of the infected specimens.

Light microscopy

After excision, the xenomas were measured and squashed for wet-mount preparations. Free fresh spores were observed and photographed using a light microscope equipped with differential interference contrast optics and coupled to a digital camera. Morphometric analysis was performed from the observation of mature spores ($n=50$) belonging to several xenomas, and the parasite was identified as a member of the phylum Microsporidia. All measurements are in micrometers.

Transmission electron microscopy

For transmission electron microscopy, excised xenomas were fragmented and fixed with 5% glutaraldehyde buffered in 0.2 M sodium cacodylate (pH 7.2) for 20–24 h, washed overnight in the same buffer, and post-fixed with 2% osmium tetroxide in the same buffer for 3 h. All of these steps were performed at 4 °C. Following dehydration in an ascending ethanol and propylene oxide series, the samples were embedded in Epon. Semi-thin sections were stained with methylene blue-Azure II. Ultrathin sections were double-contrasted with uranyl acetate and lead citrate, observed and photographed using a JEOL 100CXII TEM operated at 60 kV.

DNA extraction, amplification and sequencing

For the molecular analysis, xenomas were excised and fixed in absolute alcohol at 4 °C and the genomic DNA of approximately 5×10^6 spores extracted using a GenEluteTM Mammalian Genomic DNA Miniprep Kit (Sigma) following the manufacturer's instructions for animal tissues, with a 12 h period of incubation. The DNA was stored in 50 µl of TE buffer at –20 °C. The majority of the region coding for the SSU rRNA gene was amplified by PCR using the primers V1f (5'-CACCAAGGTTGATTCTGCC-3') (Nilsen 2000) and HG5F_rev (5'-TCACCCCCACTTGTCGTTA-3')

(Abdel-Baki et al. 2015). To amplify the 3' end of the SSU rRNA gene, the internal transcribed spacer (ITS) and the 5' end of the LSU rRNA gene, the primers HG4F (5'-CGGCTTAATTGACTCAAC-3') and HG4R (5'-TCTCCTGGTCCGTGTTCAA-3') (Gatehouse and Malone 1998) were used. PCR reactions were carried out in 50 µl reactions using 10 pmol of each primer, 10 nmol of each dNTP, 2.5 mM of MgCl₂, 5 µl 10 × Taq polymerase buffer, 1.5 units Taq DNA polymerase (Nzytech), and 3 µl of the genomic DNA. Reactions were run on a Hybaid PxE Thermocycler (Thermo Electron Corporation, Milford, MA), with initial denaturation at 94 °C for 5 min, followed by 35 cycles of 94 °C for 1 min, 50 °C for 1 min and 72 °C for 2 min. The final elongation step was performed at 72 °C for 10 min. Five-µl aliquots of the PCR products were electrophoresed through a 1% agarose 1 × tris-acetate-EDTA buffer (TAE) gel stained with ethidium bromide. The PCR products for the SSU gene and ITS region, with an approximate size of 900 bp and 1100 bp, respectively, were purified using a single-step enzymatic cleanup that eliminates unincorporated primers and dNTPs. The sequencing reactions were performed using a BigDye Terminator v1.1 kit from the Applied Biosystems kit, and were run on an ABI3700 DNA analyser (Perkin-Elmer, Applied Biosystems, Stabvida, Co., Oeiras, Portugal).

Distance and phylogenetic analysis

The forward and reverse sequence segments obtained were aligned and corrected manually using Clustal W (Thompson et al. 1994) in MEGA 5 software (Tamura et al. 2011), and ambiguous bases were clarified using corresponding ABI chromatograms.

To evaluate the relationship between *Glugea arabica* n. sp. and other microsporidians, a search was performed using the BLAST homology score for molecular and phylogenetic inferences. Fifty-two rRNA sequences belonging to fish infecting microsporidians were selected for analysis. The sequence and NCBI accession number data obtained from Genbank are as follows: *Dasyatispora levantinae* (GU183263); *Desmozoon lepeophthrii* (HM800847); *Enterospora nucleophila* (KF135645); *Glugea anomala* (AF044391); *G. atheriniae* (U15987); *G. epinephelus* (AY090038); *G. hertwigi* (GQ203287); *G. nagelia* (KJ802012); *G. pagri* (JX852026); *G. plecoglossi* (AJ295326); *G. stephani* (AF056015); *Glugea* sp. GS1 (AJ295325); *Heterosporis anguillarum* (AF387331); *Heterosporis* sp. PF (AF356225); *Heterosporis* sp. NBDP-2013a (KC137548); *Ichthyosporidium weisi* (JQ062988); *Ichthyosporidium* sp. (L39110); *Kabatana takedai* (AF356222); *K. rondoni* (FJ843105); *Loma acerinae* (AJ252951); *L. embiotocia* (AF320310); *L. morhua* (GQ121037); *L. psittaca* (FJ843104); *L. salmonae* (U78736); *Loma* sp. SVB-PE3 (HM626217); *Microsporidium cerebralis* (JQ316511); *Ovipleistophora mirandellae*

(AF356223); *O. ovariae* (AJ252955); *Microgemma carolinus* (JQ085991); *M. caulleryi* (AY033054); *M. vivaresi* (AJ252952); *Myosporidium merluccius* (AY530532); *Nucleospora salmonis* (U78176); *N. cyclopteri* (KC203457); *Obruspora papernae* (HG005137); *Pleistophora ehrenbaumi* (AF044392); *P. finisterrensis* (AF044393); *P. hippoglossoideoes* (AJ252953); *P. hypessobryconis* (GU126672); *P. typicalis* (AF044387); *Pleistophora* sp. 1 (AF044394); *Pleistophora* sp. 2 (AF044389); *Pleistophora* sp. 3 (AF044390); *Pleistophora* sp. KB-2011 (HQ703580); *Pleistophora* sp. LM-2014 (KF830721); *Potaspura moraphis* (EU534408); *Pseudoloma neurophilia* (AF322654); *Spraguea americana* (AF056014); *S. americana* (AY465876); *S. lophii* (AF033197); *S. gastraphysus* (GQ864443); *Tetramicra brevifilum* (AF364303). *Vairimorpha necatrix* (Y00266) was used as outgroup.

The alignment was performed using ClustalW (Thompson et al. 1994) in MEGA 5.05 software (Tamura et al. 2011), with an opening gap penalty of 10 and a gap extension penalty of 4 for both pairwise and multiple alignments. After trimming the LSU rRNA 3'-end, the resulting alignment comprised 2136 informative characters in the final dataset. Subsequent phylogenetic and molecular evolutionary analyses were conducted in MEGA 5.05, using maximum likelihood (ML), neighbor-joining (NJ) and maximum parsimony (MP) methodologies. For ML, the general time reversible substitution model with 4 gamma distributed rate variation among sites was performed. For NJ, a Kimura 2-parameter substitution model with gamma distribution (shape parameter = 1.4) was performed. For MP, the close neighbour interchange heuristic option with a search factor of 1 and a random initial tree addition of 500 replicates was performed. All positions with less than 75% site coverage were eliminated from all trees and the bootstrap consensus tree was inferred from 500 replicates for ML, NJ and MP.

Distance estimation was carried out in MEGA 5.05, using the p-distance model distance matrix for transitions and transversions, with all ambiguous positions removed for each sequence pair.

Results

Small blackish xenomas were macroscopically observed adhering to the intestinal epithelium of the teleost fish *Epinephelus polyphekadion* (Fig. 1A, B). These structures contained numerous ellipsoidal spores, whose morphology, when observed in wet-mount preparations, was identified as belonging to the phylum Microsporidia (Fig. 1C). The parasite, herein named *Glugea arabica* n. sp. is described based on ultrastructural and molecular features, with the following taxonomic position:

Phylum Microsporidia Balbiani, 1882

Class Marinospordia Vossbrinck and Debrunner-Vossbrinck, 2005

Order Glugeida Issi, 1986

Family Glugeidae Thélohan, 1892

Genus *Glugea* Thélohan, 1891

Glugea arabica n. sp.

Diagnosis

Xenomas: Spherical and measuring up to 1.0 mm, formed by a wall encircling a hypertrophic cell displaying numerous parasitophorous vacuoles (Fig. 1A, B) containing a variable number of ellipsoidal spores (Fig. 1C). The periphery of the xenomas consisted of a refractile capsule composed of laminar structures (Fig. 1A). Young developmental stages were observed intermingled with the parasitophorous vacuoles containing mature spores (Fig. 1A, B). In the cytoplasm of the xenomas, the ramified hypertrophic nucleus was observed, as well as numerous mitochondria and small vesicles surrounding the parasitophorous vacuoles (PV's) (Fig. 2A).

Young developmental stages: Some multinucleate meronts encircled by a fine wall were observed among the PV's containing other developmental stages (Figs. 1A, B, 2B). Several young stages bounded by a layer of electron dense material outside the plasmalemma were observed intermingled

with the PV's. Within the latter, several sporoblasts were dispersed in the episporal space (Fig. 2C–E). There are about 10–20 spores in each PV's and apparently episporal space was originated by the host cell.

Spores: Unfixed spores were monomorphic, ellipsoidal and measured 6.3 ± 0.3 (5.9–6.6) \times 3.3 ± 0.4 (2.9–3.7) μm ($n=50$) (Fig. 1C). The spore wall was ~ 105 nm thick, corresponding to an electron-dense exospore (~ 30 nm thick) and an electron-lucent endospore (~ 75 nm thick) (Fig. 3C–E). Spores were uninucleate, with the nucleus located between the apical polaroplast and the posterior vacuole (Figs. 3A, B, 4). The anchoring disc appeared at an excentric position and extended to form the polar filament, which displayed an angle of tilt of $32 \pm 5^\circ$ (Fig. 3D). The polar filament measured 110–130 nm in diameter (Fig. 3E inset), was isofilar and consisted of 27–29 coils with an angle of tilt of $62 \pm 7^\circ$ and organized in three to four rows that surrounded the posterior vacuole (Fig. 3E). The latter occupied about one third of the total volume of the spore and contained an irregular contour surrounding a light granulo-fibrillar matrix where the posterosome was located (Fig. 3B). The polaroplast appeared surrounding the initial portion of

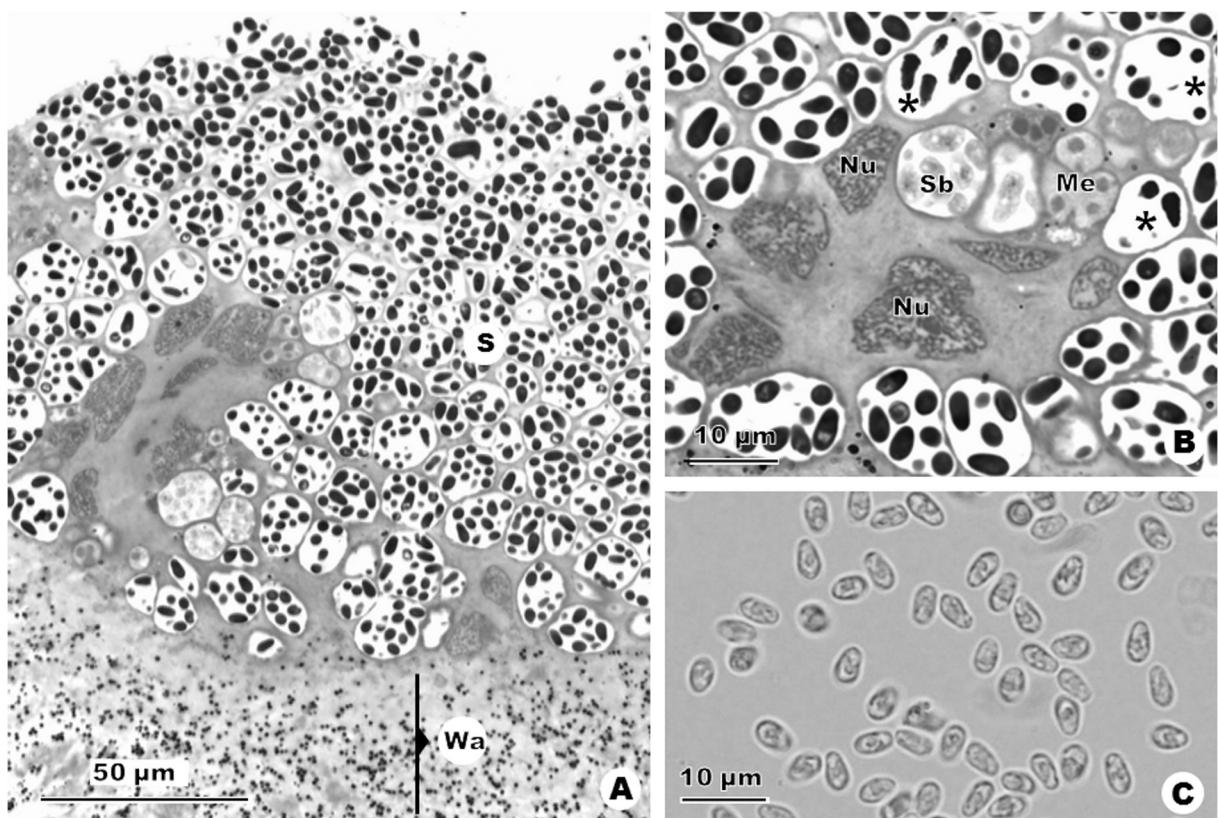


Fig. 1. (A–C) Light microscopy micrographs of some developmental stages of the microsporidium *Glugea arabica* n. sp. infecting the teleostean *Epinephelus polyphekadion* from the Arabian Red Sea coast. (A) Semithin section of part of a xenoma showing numerous parasitophorous vacuoles containing spores. Just near the xenoma wall (Wa) some developmental stages are present. (B) Detail of a xenoma showing some parasitophorous vacuoles (*) containing mature spores, surrounding some meronts (Me) and sporoblasts (Sb). (C) Several fresh mature spores observed in differential interference contrast microscopy.

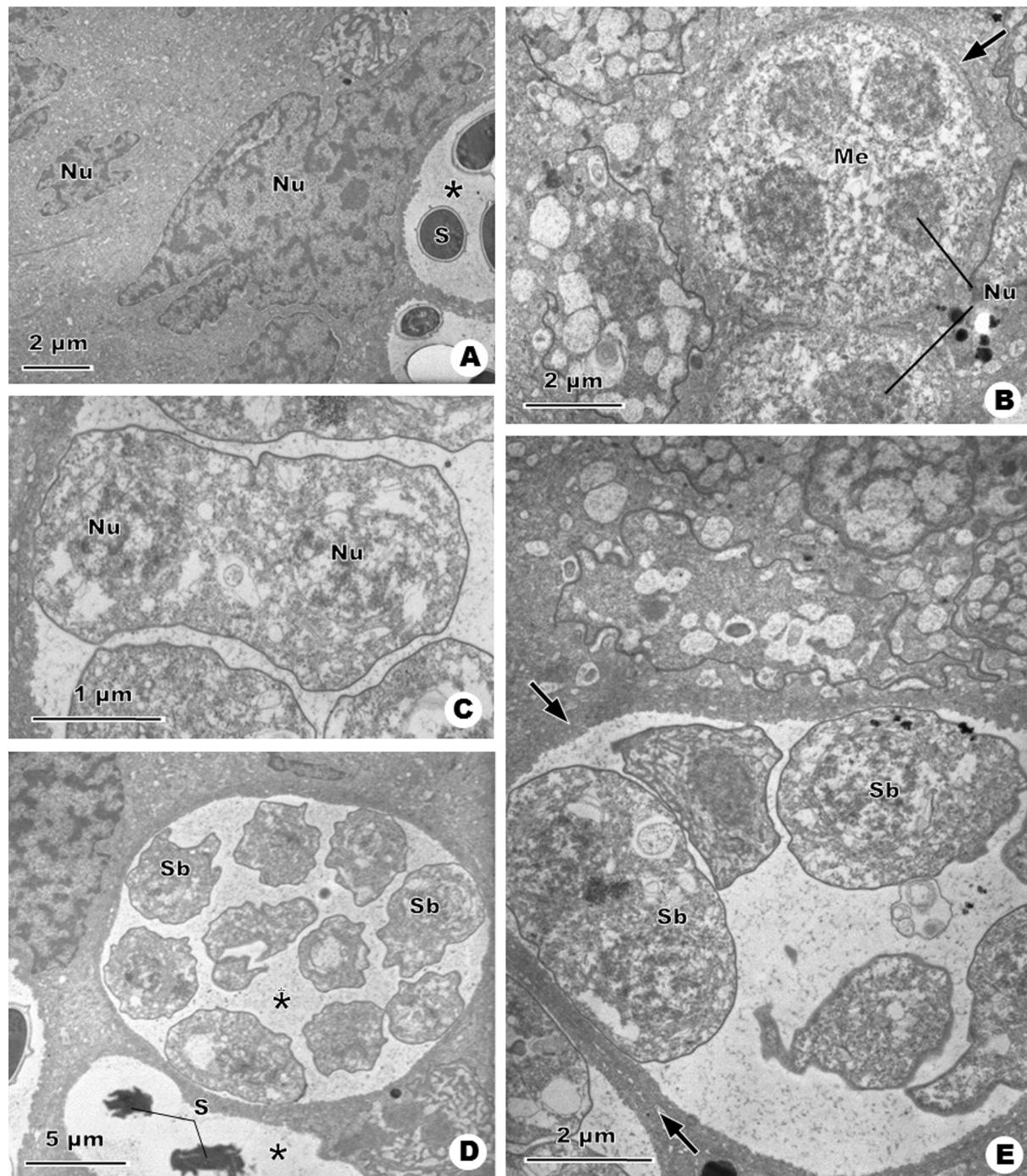


Fig. 2. (A–E) Transmission electron micrographs of some developmental stages of the microsporidian *Glugea arabica* n. sp. infecting the teleostean *Epinephelus polyphekadion* from the Arabian Red Sea coast. (A) Internal periphery of the xenoma, showing the host cell nuclei hypertrophic and branched (Nu) and parasitophorous vacuoles (*) with spores (S). (B) Meront in division (Me) encircled by a wall (arrow). Nuclei (Nu). (C) Binucleated sporoblast mother cell in division showing the nuclei (Nu). (D and E) Parasitophorous vacuoles (*) showing some early sporoblasts (Sb) in differentiation and matures spores (S) encircled by dense wall (arrows).

the polar filament (manubrium) and it displayed a fine structural organization characterized by numerous juxtaposed lamellae, while the posterior region presented an electrodense vesicular arrangement (Fig. 3C, D).

Type host: The camouflage grouper *Epinephelus polyphekadion* (fam. Serranidae).

Type locality: Rea Sea coast of Saudi Arabia, near the city of Jeddah ($21^{\circ}31'N$, $39^{\circ}13'E$).

Site of infection: Intestinal wall.

Prevalence: 15/24 (62.5%).

Etymology: The specific epithet “*arabica*” derives from the name of the country.

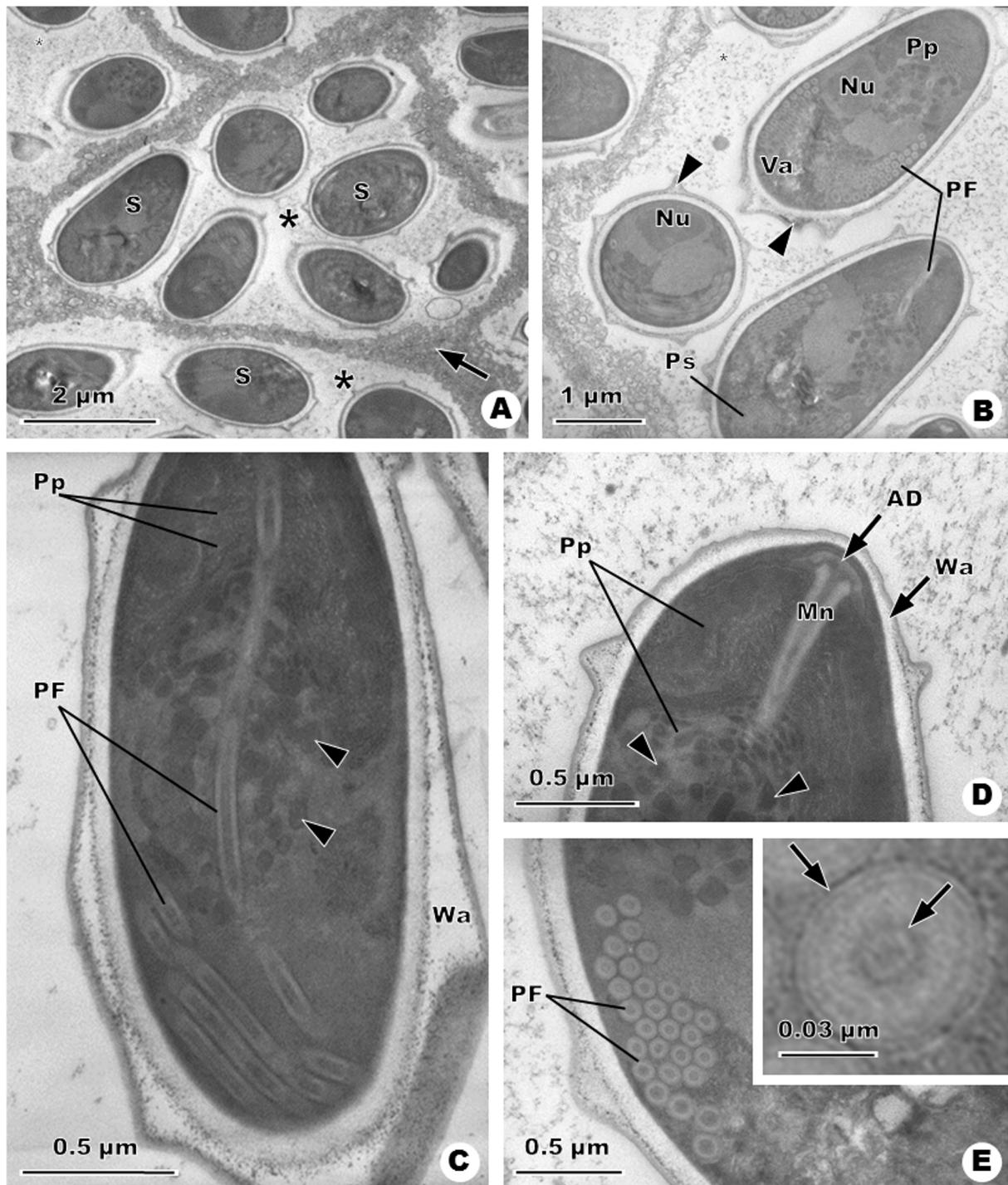


Fig. 3. (A–E) Transmission electron micrographs of mature spores of the microsporidian *Glugea arabica* n. sp. infecting the teleostean *Epinephelus polyphekadion* from the Arabian Red Sea coast. (A) Parasitophorous vacuole (*) showing several mature spores (S) sectioned at different levels. The parasitophorous vacuole wall (arrow) with a very irregular outline and with different thickness. (B) Longitudinal and transverse sections of the spores contained within the episporal space of the parasitophorous vacuole (*) showing the wall (Wa), nucleus (Nu), polar filament (PF), posterosome (Ps) inside the posterior vacuole (Va). (C) Longitudinal section of a spore showing a projection of the polar filament (PF) just to the basal portion of the spore, the polaroplast (Pp) well structured in anterior region and a vesicular organization in posterior portion (arrowheads). Wall (Wa). (D) Detail of the apical region of the spore showing the spore wall (Wa), the anchoring disc (AD) in continuity with manubrium (Mn), polaroplast (Pp) and some posterior portion of polaroplasto (arrowheads). (E) Details of the 28 transverse polar filament sections (PF). Inset: Detail of a transverse polar filament section showing the internal organization with some evident circular layers (arrows).

Type specimens: [Upon acceptance]. One glass slide with semithin sections containing several developmental stages of microsporidia of the hapantotype deposited in the Type Slide Collection of the Laboratory of Animal Pathology at the Interdisciplinary Centre of Marine and Environmental Research, Porto, Portugal, reference CIIMAR 2015.8, and DNA sequence corresponding to the complete SSU rRNA gene, ITS and partial LSU rRNA gene 1763 bp long (accession number KT005391) deposited in GenBank database.

Molecular and phylogenetic analysis

The complete SSU rRNA gene sequence + ITS region and partial LSU rRNA gene of *Glugea arabica* n. sp. was obtained through amplification with the primer pairs V1f/HG5F_rev and HG4F/HG4R. Alignment of these sequences resulted in the construction of a sequence of consensus with 1763 bp in length and containing a GC content of 52.6%. The sequence was deposited in GenBank database under the accession number (KT005391).

A Blast search for similar sequences to the 16S rRNA gene confirmed relationships to other microsporidia that parasitize fish, with highest identity score for *Glugea nagelia* (KJ802012) and *Glugea epinephelus* (AY090038). Fifty-two rRNA sequences belonging to fish infecting microsporidians from the five phylogenetic groups acknowledged for this phylum, were selected and aligned. The phylogenetic analysis showed that *Glugea arabica* n. sp. was most similar to *Glugea* and *Loma* sp., as according to the classification proposed by Lom and Nilsen (2003). For pairwise comparisons among the rRNA genes, a second alignment was performed and contained all *Glugea* spp. All ambiguous positions were removed for each sequence pair, resulting in a total of 1895 positions in the final dataset. The minimum genetic distance (p-distance) of 0.6% was observed for both *Glugea nagelia* (KJ802012) and *G. epinephelus* (AY090038). All other analysed sequences presented genetic distance equal to or greater than 1.7% (Table 1). In all the phylogenetic analyses (ML, NJ, MP), there was strong evidence for a solid clade, with a bootstrap of 100%, 100% and 99% respectively, composed by *Glugea nagelia*, *G. epinephelus* and *Pleistophora* sp. (this last species is yet unpublished) (Fig. 5).

Discussion

The morphological and ultrastructural observations of the xenomas, developmental stages and mature spores revealed all the structures typical of the parasites belonging to the Phylum Microsporidia (Lom and Dyková 1992; Larsson 1999; Lom and Nilsen 2003). Despite the formation of parasitophorous vacuoles being a common characteristic of not only *Glugea*, but also of the genera *Loma*, *Pleistophora* and *Pseudoloma*, several other aspects of the parasite's

Table 1. Comparison of some rDNA sequences: percentage of identity (top diagonal) and pairwise distance (bottom diagonal) obtained by p-distance.

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
(1) <i>Glugea arabica</i> n. sp. (KT005391)	—													
(2) <i>Glugea nagelia</i> (KJ802012)	0.006	—												
(3) <i>Glugea epinephelus</i> (AY090038)	0.006	0.007	—											
(4) <i>Pleistophora</i> sp. (KF830721)	0.017	0.018	0.019	—										
(5) <i>Glugea stephani</i> (AF056015)	0.070	0.071	0.074	0.071	—									
(6) <i>Glugea atheriniae</i> (U15987)	0.080	0.081	0.081	0.076	0.005	—								
(7) <i>Glugea pagri</i> (JX852026)	0.082	0.083	0.083	0.081	0.019	0.016	—							
(8) <i>Glugea hertwigi</i> (GQ203287)	0.101	0.101	0.080	0.078	0.003	0.005	0.018	—						
(9) <i>Glugea plecodossi</i> (AJ295325)	0.104	0.104	0.078	0.077	0.007	0.008	0.016	0.011	—					
(10) <i>Glugea</i> sp. GS1 (AJ295325)	0.104	0.104	0.077	0.076	0.005	0.007	0.014	0.088	0.005	—				
(11) <i>Loma psittaca</i> (FJ843104)	0.107	0.109	0.110	0.110	0.091	0.101	0.094	0.102	0.087	0.086	—			
(12) <i>Glugea anomala</i> (AF044391)	0.108	0.108	0.083	0.079	0.009	0.000	0.022	0.015	0.013	0.013	0.104	—		
(13) <i>Pleistophora finisterrensis</i> (AF044393)	0.109	0.109	0.080	0.077	0.008	0.009	0.017	0.012	0.012	0.010	0.087	0.013	—	
(14) <i>Loma acerinae</i> (AJ252951)	0.100	0.102	0.102	0.102	0.091	0.090	0.092	0.082	0.079	0.077	0.096	0.084	—	

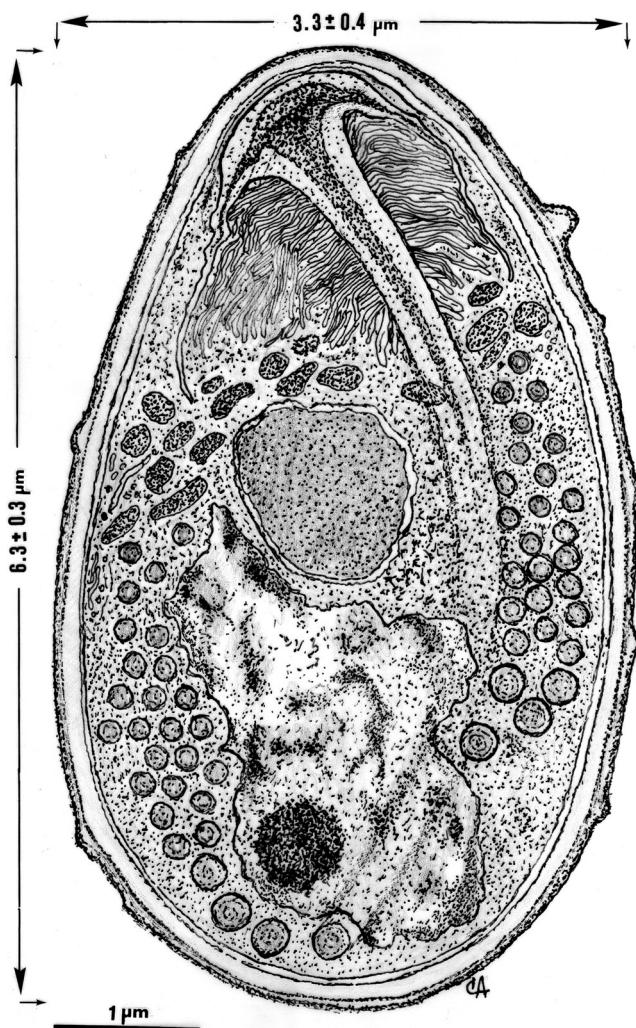


Fig. 4. Schematic drawing of a mature spore of *Glugea arabica* n. sp. obtained based in different longitudinal serial ultrathin sections showing the internal organization and some measurements.

development are in close agreement with those more specific of the genus *Glugea* (Lom 2008).

Members of the genus *Glugea* have been described from both marine and freshwater fish in several different geographic locations, and frequently induce the development of large whitish or blackish xenomas in the musculature and connective tissue of visceral organs, namely in the intestine wall (Abdel-Baki et al. 2015; Canning et al. 1982; Jithendran et al. 2011; Lovy et al. 2009; Vagelli et al. 2005).

Comparing the morphological and ultrastructural characteristics, host specificity and habitat amongst the 27 recognized species of the genus *Glugea* (Lom and Dyková 1992; Lom 2002; Vagelli et al. 2005; Wu et al. 2005; Su et al. 2014), it is noted that *G. capverdensis*; *G. hertwigi*; *G. shiplei*; *G. schulmani* and *G. stephani* also parasitize the intestine of marine hosts, but present no other gross similarity to *Glugea arabica* n. sp. Recently, Abdel-Baki et al. (2015) described the species *Glugea nagelia* from the intestinal wall of the yellowfin hind *Cephalopholis hemistictos* caught in this same

geographic region of the Red Sea coast off Saudi Arabia. Our species possesses some morphometric similarities to *G. nagelia*, namely the size of the spores and the number of coils of the polar filament. These species differ in the type of xenomas developed, however: whitish (*G. nagelia*) and blackish (*G. arabica*) and there is a genetic distance of 0.6%. Other than these species, only *Pleistophora* spp. (Abdel-Ghaffar et al. 2009, 2011, 2012; Morsy et al. 2012) and *Microsporidium aurata* (Morsy et al. 2013) have been described from the Red Sea.

Some references are also available for microsporidian parasites that infect fish of the genus *Epinephelus* (Serranidae). In the intestinal epithelium of *E. chlorostignei*, a microsporidian parasite classified as *Pleistophora* sp. was morphologically and ultrastructurally described (Abdel-Ghaffar et al. 2009); and a *Glugea* species was identified in the subcutaneous tissue and visceral organs of the greasy grouper *E. tauvina* caught from the coastal waters off Muttukkadu, near Chennai (India), a *Glugea* species was identified (Jithendran et al. 2011). *Glugea epinephelus* was morphological and molecularly described infecting the abdominal cavity of *Epinephelus akaara* in a Chinese aquaculture facility (Wu et al. 2005). An interesting feature that is shared between these three species, besides host family, is the blackish tone of the xenomas.

Analysing the molecular data presently available for Microsporidia, GenBank accounts for four rRNA sequences of these parasites infecting fish in the Red Sea; one of which is *Glugea nagelia* (Abdel-Baki et al. 2015). *Pleistophora aegyptiaca* (JF514548), *P. pagri* (JF797622) and *Microsporidium aurata* (KF022044) were not considered for analysis because their sequences are very short (650, 249 and 538 bp respectively) and, therefore, do not allow strong phylogenetic inferences with rRNA sequences that present a high similarity score according to BLAST homology. On the other hand any of these sequences belongs to the genus *Glugea*.

Analysing the molecular and phylogenetic data obtained, it is noted the formation of a clade is suggested, containing *G. arabica*, *G. nagelia* and *G. epinephelus*, and a *Pleistophora* sp. sequenced from a fish hosts of the Red Sea fauna (KF830721). This position was concordant and well-supported in all phylogenetic trees performed. Similar to what is being shown for other parasitic taxa, such as Myxozoa, microsporidian phylogeny also appears to be influenced by evolutionary signals that relate to the hosts habitat, as well as to the organ or tissue of infection (Eszterbauer 2004; Fiala 2006). Considering the most representative genera of the phylum Microsporidia, *Glugea* appear to have a preference for the smooth musculature and/or the connective tissues of visceral organs, and more frequently of the intestinal wall; *Pleistophora* infect the skeletal muscle; *Microgemma* the liver; *Spraguea* the nervous system; several *Loma* the branial filaments; and *Ovipleistophora* the gonads (Lom 2008). Most of these genera are monophyletic, with the exception of *Glugea* and *Loma*. Further sequencing of the rRNA genes

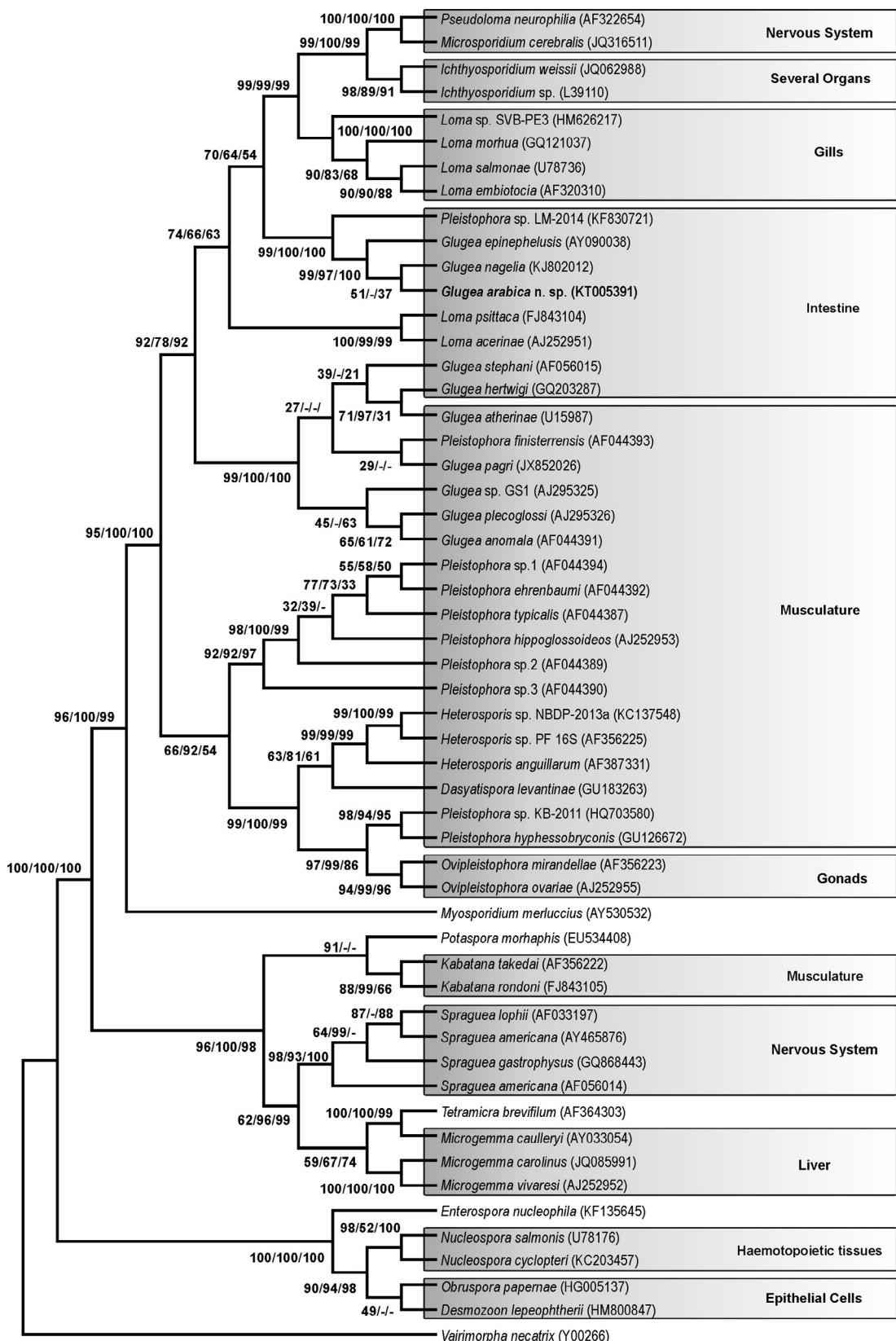


Fig. 5. Maximum Likelihood tree of the SSU rDNA sequences of *Glugea arabica* n. sp. and other selected microsporidian species. The numbers on the branches are bootstrap confidence levels on 500 replicates for ML/NJ/MP trees. There were a total of 1211 positions in the final dataset. GenBank accession numbers in parentheses after the species name.

of Microsporidia, especially of *Glugea*, will probably lead to some taxonomic changes, as well as to the recognition of further evolutionary signals influencing the phylogeny of this parasitic group.

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