Folia Parasitologica 2015, 62: 007
doi: 10.14411/fp.2015.007

Folia Parasitologica
http://folia.paru.cas.cz

Research Article OPEN ACCESS

Ultrastructure and phylogeny of *Glugea nagelia* sp. n. (Microsporidia: Glugeidae), infecting the intestinal wall of the yellowfin hind, *Cephalopholis hemistiktos* (Actinopterygii: Serranidae), from the Red Sea

Abdel-Azeem S. Abdel-Baki[1,2], **Saleh Al-Quraishy**[1], **Sónia Rocha**[3,4], **Mohamed A. Dkhil**[1,5], **Graça Casal**[3,6] and **Carlos Azevedo**[1,3,4]

[1] Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia;

[2] Zoology Department, Faculty of Science, Beni-Suef University, Egypt;

[3] Laboratory of Animal Pathology, Interdisciplinary Centre of Marine and Environmental Research, University of Porto, Porto, Portugal;

[4] Laboratory of Cell Biology, Institute of Biomedical Sciences, University of Porto, Porto, Portugal;

[5] Department of Zoology and Entomology, Faculty of Science, Helwan University, Cairo, Egypt;

[6] Department of Sciences, High Institute of Health Sciences – North, Gandra, Portugal

Abstract: A new microsporidian species of the genus *Glugea* Thélohan, 1891 parasitising the marine teleost fish *Cephalopholis hemistiktos* Rüppell, collected from the Red Sea in Saudi Arabia, is described on the basis of microscopic and molecular procedures. Spherical and whitish xenoma were observed adhering to the intestinal wall. The numerous spores contained within these xenoma, were ovoid to pyriform and measured 4.3–6.0 µm (5.1 µm) in length and 1.8–2.9 µm (2.2 µm) in width. The spore's wall was composed of two thick layers, which were thinner in the area contacting the anchoring disk. The latter appeared at the spore's anterior pole, in an eccentric position to the longitudinal axis. A lamellar polaroplast surrounded the uncoiled portion of the polar filament projected to the basal region of the spore, giving rise to 26–29 turns with winding from the base to the anterior zone of the spore. The posterior vacuole, located at the spore's posterior pole, and surrounded by the polar filament coils, was irregular and composed of light material. Molecular analysis of the rRNA genes, including the ITS region, was performed using maximum parsimony, neighbour-joining and maximum likelihood methods. The ultrastructural features observed, combined with the phylogenetic data analysed, suggest this parasite to be a new species of the genus *Glugea*. This is the first species of this genus to be reported from Saudi Arabia and is herein named *Glugea nagelia* sp. n.

Keywords: fish parasite, microsporidian, fine structure, rRNA genes

Microsporidia Balbiani, 1882 are obligate intracellular parasites of medical and commercial importance. They are characterised by a significant reduction in, or even absence of, the cellular components typical of eukaryotes, such as mitochondria, Golgi apparatus and flagella (Corradi and Selman 2013). Ubiquitous in nature, microsporidians infect a wide taxonomical range of hosts, namely protists, arthropods and vertebrates, including humans (Weiss and Vossbrinck 1998, Larsson 1999, Vossbrinck and Debrunner-Vossbrinck 2005). The majority of species, however, have been described from insect and fish hosts (Cali and Takvorian 2003).

At present, the phylum Microsporidia comprises over 1 300 species distributed among 160 genera, 18 of which refer to fish-infecting species (Lom and Nilsen 2003). Of the latter, 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). Descriptions are mainly based on cellular structure, life cycle and host specificity (Corradi and Keeling 2009). More recently, molecular analysis has also become a requisite for the understanding of microsporidian taxonomy and phylogeny (Weiss and Vossbrinck 1999, Vossbrinck and Drebunner-Vossbrinck 2005). In the case of *Glugea*, however, GenBank provides rRNA sequences for only eight species, which means that only a limited data are available for reliable understanding of the phylogeny of this genus.

Address for correspondence: A.-A.S. Abdel-Baki, Zoology Department, College of Science, King Saud University, 11451 Riyadh, Saudi Arabia.
Phone: +96614675754; Fax: +96614678514; E-mail: azema1@yahoo.com

Zoobank number for article: urn:lsid:zoobank.org:pub:C4E7EC38-30CB-4BF3-A841-D8B9827E44E9

Very little is known about the distribution and diversity of microsporidian parasites of the Red Sea fishes. Research on these parasites is restricted to the description of just three species from the entire extent of the Red Sea coastline, all of which were described from Egypt. These species are *Pleistophora pagri* Morsy, Abdel-Ghaffar, Mehlhorn, Bashtar, Abdel-Gaber, 2012 from *Pagrus pagrus* Linnaeus, *Pleistophora aegyptiaca* Abdel-Ghaffar, Bashtar, Morsy, Mehlhorn, Al Quraishy, Al-Rasheid, Abdel-Gaber, 2012 from *Saurida tumbil* Bloch and *Microsporidium aurata* Morsy, Bashtar, Abdel-Ghaffar, Al-Quraishy, 2013 from *Sparus aurata* Linnaeus (see Abdel-Ghaffar et al. 2012, Morsy et al. 2012, 2013).This paper thus represents the first report of the occurrence of a microsporidian infecting fish in the Red Sea outside of Egypt. Using ultrastructural and molecular data, a new *Glugea* is described.

MATERIALS AND METHODS

Host and parasite sampling

One hundred freshly caught yellowfin hind fish, *Cephalopholis hemistiktos* Rüppell (Actinopterygii: Serranidae) (common name nagel fish), were collected from the boat landing site in Jeddah, Saudi Arabia, throughout a period from November 2012 to November 2013. Specimens averaged ~20 cm in length. Upon necropsy, macroscopic observations revealed the presence of several xenoma adhering to the intestinal wall and the body cavity of some of the specimens. Xenoma were measured and preserved in 4% glutaraldehyde for electron microscopical study and in 95% ethanol for molecular study.

Light and electron microscopy

For observation of fresh spores, the xenoma was compressed for the preparation of smears, which were then observed and photographed using differential interference contrast (DIC) optics coupled to a digital camera. Morphometric analysis was performed on mature spores (n = 30) from different cysts, with all measurements obtained using a digital scale bar and recorded in micrometres.

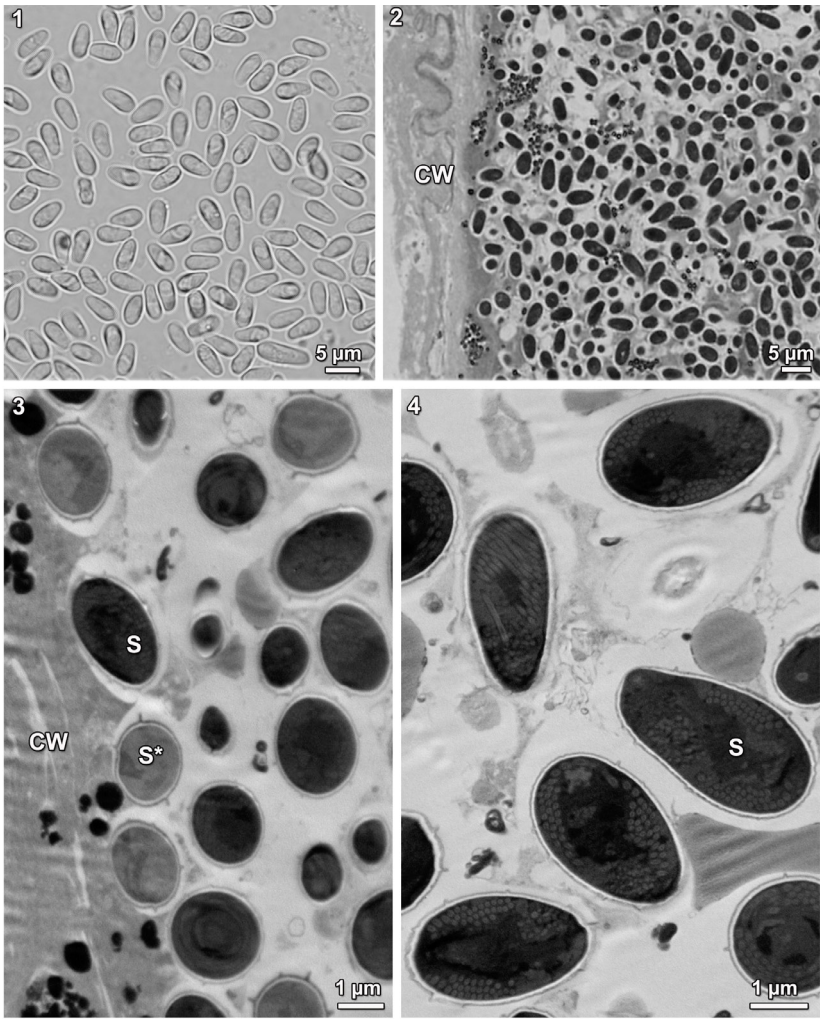
For transmission electron microscopy, xenoma was excised and fixed with 4% glutaraldehyde buffered in 0.2 M sodium cacodylate (pH 7.2) for 24 h, washed overnight in the same buffer and post-fixed with 2% osmium tetroxide in the same buffer for 3 h. All of these steps were performed at 4 °C. Following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

DNA preparation and phylogenetic analysis

For molecular analysis, cysts were fixed in 95% ethanol at 4 °C and the genomic DNA of approximately 5×10^6 spores was extracted using a GenElute™ Mammalian Genomic DNA Mini prep Kit (Sigma) following the manufacturer's instructions for animal tissue, 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'-CACCAGGTTGATTCTGCC-3') (Nilsen 2000) and HG5F_rev (5'-TCACCCCACTTGTCGTTA-3') (designed for this study). 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'-CGGCTTAATTTGACTCAAC-3') and HG4R (5'-TCTCCTTGGTCCGTGTTTCAA-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 mM of $MgCl_2$, 5 µl 10×Taq polymerase buffer, 1.25 units Taq DNA polymerase and 3 µl of the genomic DNA.

Reactions were run on a HybaidPxE 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 1 100 bp, respectively, were purified using a single-step enzymatic clean-up (NZYGelpure kit – Nzytech) that eliminates unincorporated primers and dNTPs and subsequently sequenced. The sequencing reactions were performed using a Big Dye 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).

For phylogenetic analysis, 29 microsporidian SSU rRNA sequences were extracted from GenBank according to their BLAST homology score (Altschul et al. 1990). These were: *Dasyatispora levantinae* Diamant, Goren, Yokeş, Galil, Klopman, Huchon, Szitenberg et Karhan, 2012; *Glugea anomala* Nilsen, 1999; *G. atherinae* Da Silva, Slemenda, Ditrich et Pieniazek, 1994; *G. hertwigi* Lovy, Kostka, Dykova, Arsenault, Peckova, Wright et Speare, 2009; *G. plecoglossi* Bell, Aoki et Yokoyama, 2001; *G. stephani* Pomport-Castillon, de Jonckheere et Romestand, 1998; *Glugea* sp. GS1 of Bell et al. (2001); *Glugea* sp. of Wu et al. (2005); *Heterosporis anguillarum* Tsai, Yang, Lo et Wang, 2002; *Heterosporis* sp. PF of Pekkarinen et al. (2002); *Ichthyosporidium weisii* Sanders, Myers, Tomanek, Cali, Takvorian et Kent, 2012; *Loma acerinae* Cheney, Lafranchi-Tristem et Canning, 2000; *L. psittaca* Casal, Matos, Teles-Grilo et Azevedo, 2009; *Microsporidium cerebralis* Jones et Prosperi-Porta, 2012; *M. cypselurus* Yokoyama, Lee et Bell, 2002; *Microsporidium* sp. STF unpublished sequences of Refardt and Pugovkin submitted in 2003; *Ovipleistophora mirandellae* Nilsen, 2001; *O. ovariae* Cheney, Lafranchi-Tristem et Canning, 2000; *Pleistophora aegyptiaca*, *P. ehrenbaumi* Nilsen, Endresen et Hordvik, 1999, *P. finisterrensis* Nilsen, Endresen et Hordvik, 1998; *P. hippoglossoideos* Cheney, Lafranchi-Tristem et Canning, 2000; *P. hyphessobryconis* Sanders et Kent, 2009; *P. mulleri* Ironside, Wilkinson et Rock, 2008; *P. typicalis* Nilsen, Endresen et Hordvik, 1998; *Pleistophora* sp. 1–3 of Nilsen et al. (1998) and *Pleistophora* sp. unpublished sequences of Li et al. submitted in 2011. The sequences *Potaspora morhaphis* Casal, Matos, Teles-Grilo et Azevedo, 2008 and *Spraguea* sp. of Miwa et al. (2011) were used as outgroup. The alignment was performed using Clustal W (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.



Figs. 1–4. Light and transmission electron micrographs of *Glugea nagelia* sp. n. from the intestine of *Cephalopholis hemistiktos*. **Fig. 1.** Numerous fresh spores observed under DIC optics. **Fig. 2.** Semithin section of a xenoma showing its wall surrounding a matrix containing numerous spores. **Fig. 3.** Ultrathin section showing the periphery of a xenoma: note the cyst wall composed of host-derived capsule wall and the presence of mature spores alongside almost immature spores. **Fig. 4.** Ultrathin section of several spores sectionned at different levels and displaying their internal organization. *Abbreviations*: CW – cyst wall; S – mature spores; S* – immature spores.

After trimming off the LSU rRNA 3'-end and excluding sites that could only be aligned ambiguously, the resulting alignment comprised 1 897 informative characters in the final dataset. Subsequent phylogenetic and molecular evolutionary analyses were conducted in MEGA 5.05, using Maximum Parsimony (MP), Neighbour-Joining (NJ) and Maximum Likelihood (ML) methodologies. Maximum parsimony analysis was performed using the close neighbour interchange heuristic option with a search factor of 1, and a random initial tree addition of 1 000 replicates. Neighbour-Joining analysis was performed using a Kimura 2-parameter substitution model with gamma distribution (shape parameter = 1.4). Maximum likelihood analysis was performed using the general time reversible substitution model with 4 gamma distributed rate variation among sites.

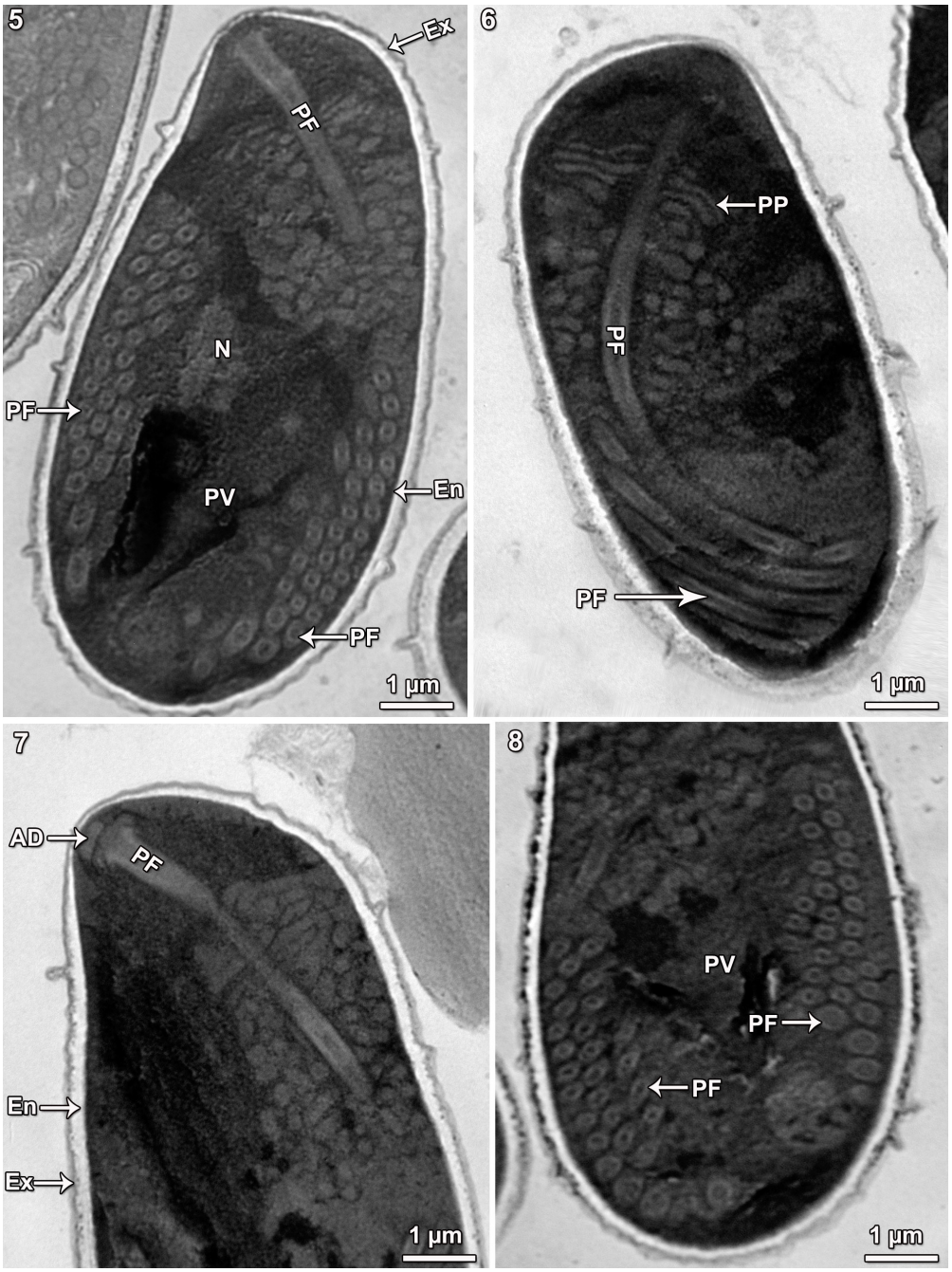
All positions with less than 50% site coverage were eliminated from all trees, resulting in a total of 1 766 positions in the final dataset. The bootstrap consensus tree was inferred from 500 replicates for MP, NJ and ML. A second alignment was performed for the SSU rDNA sequence of the case isolate and for those SSU rDNA sequences presenting the highest similarity scores in BLAST and clustering in the same clade of the case isolate, resulting in a total of 1 883 positions in the final dataset. Distance estimation was carried out in MEGA 5.05, using the Kimura 2-parameter model distance matrix for transitions and transversions, with all ambiguous positions removed for each sequence pair.

RESULTS

Glugea nagelia sp. n. Figs. 1–10

Zoobank number for species:
urn:lsid:zoobank.org:pub:C4E7EC38-30CB-4BF3-A841-D8B9827E44E9

Description: Spherical xenoma, about 2 mm in diameter, forming whitish masses adhering to intestinal wall and encapsulated by a thick and host-derived capsule wall. Development of xenoma appears synchronous and cytoplasm contains many monomorphic mature spores (Figs. 1–4). No other developmental stages observed. Mature spores ovoid to pyriform, about 4.3–6.0 (5.1) µm long and 1.8–2.9



Figs. 5–8. Transmission electron micrographs of *Glugea nagelia* sp. n. from the intestine of *Cephalopholis hemistiktos*. **Fig. 5.** Longitudinal section of a spore showing its wall composed of two layers – endospore and exospore, polar filament coiled around the posterior vacuole, and central nucleus. **Fig. 6.** Spore showing polar filament coils in slightly oblique longitudinal section, as well as polaroplast surrounding the uncoiled portion of that structure. **Fig. 7.** Ultrastructure detail displaying the spore wall composed by two thick layers: endospore and exospore. The wall appears thinner in the area contacting the anchoring disk from which the polar filament extends. **Fig. 8.** Ultrastructural detail of the polar filament coiled around the posterior vacuole. *Abbreviations*: AD – anchoring disk; En – endospore; Ex – exospore; N – nucleus; PF – polar filament; PP – polaroplast; PV – posterior vacuole.

(2.2) µm (n = 30) wide (Fig. 1). Spore wall thick, except for area of anterior pole with anchoring disk. Wall double-layered, composed of electron-dense exospore and electron-lucent endospore of approximately same thickness, displaying some protuberances (Figs. 5–7). Subterminal anchoring disc appears eccentric to spore longitudinal axis (Figs. 5, 7). Uncoiled portion of polar filament surrounded by lamellar polaroplast at spore anterior pole (Figs. 5–7). Polar filament isofilar with 26–29 coils arranged in three rows at posterior pole, winding from basal to anterior zone of spore (Figs. 5, 8). Nucleus positioned between polaroplast and posterior vacuole (Fig. 5). Posterior vacuole surrounded by polar filament coils, irregular and containing masses of denser material (Figs. 5, 6, 8). Posterosome never observed.

Type host: *Cephalopholis hemistiktos* (Rüppell) (Actinopterygii: Serranidae).

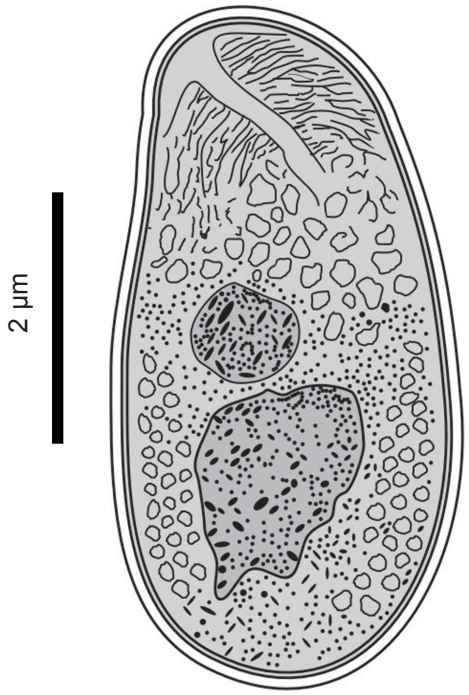


Fig. 9. Schematic drawing displaying a longitudinal section of a spore of *Glugea nagelia* sp. n. from the intestinal wall of the marine teleost *Cephalopholis hemistiktos* collected in the Red Sea, reporting its overall internal organisation.

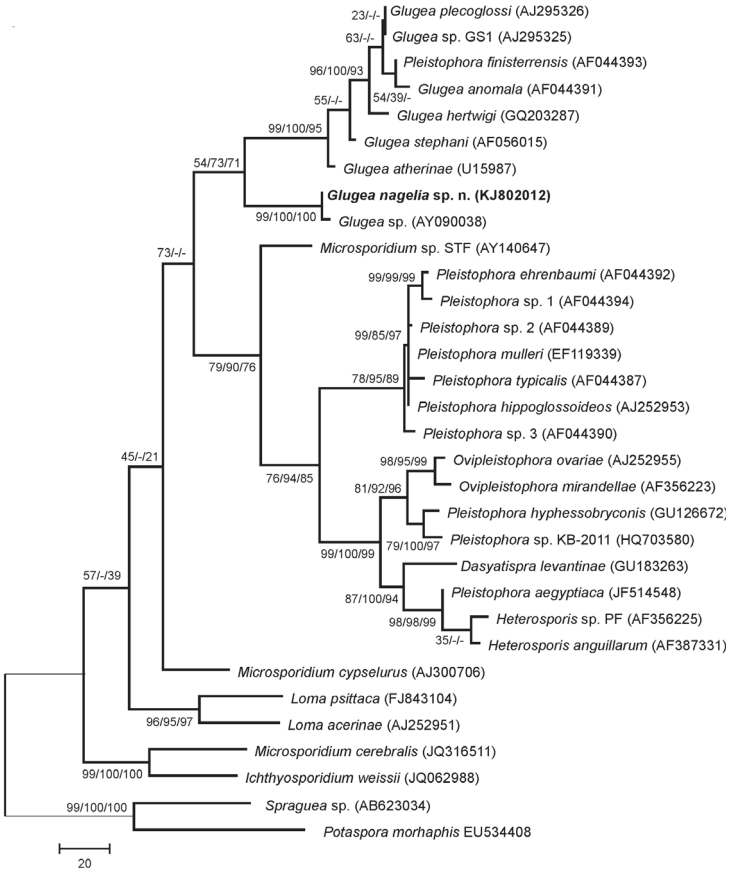


Fig. 10. Maximum parsimony tree of the SSU rDNA sequences of *Glugea nagelia* sp. n. and other selected microsporidian species. The numbers on the branches are bootstrap confidence levels on 500 replicates forMP/ NJ/ML trees. There were a total of 1 766 positions in the final dataset. GenBank accession numbers in parentheses after the species name; scale is given under the tree.

Type locality: The Red Sea coast off Jeddah (21°31'N; 39°13'E), Saudi Arabia.

Site of infection: Intestinal wall.

Prevalence of infection: 10% (n = 100 specimens).

Type material: DNA sequence corresponding to the complete SSU rRNA gene, ITS and partial LSU rRNA gene 1 782 bp long (accession number KJ802012).

Etymology: The specific epithet 'nagelia' derives from the local Arabic name of the host species (Nagel).

Molecular characterisation and phylogeny

Pairwise distances among the SSU rRNA sequences revealed that *Glugea nagelia* sp. n. exhibits the highest similarity to *Glugea* sp. from *Epinephelus awoara* Temminck et Schlegel (99.5%), *Glugea stephani* (92.5%), *G. atherinae* (91.7%), *G. hertwigi* (88.7%), *G. anomala* (88.1%), *Glugea* sp. GS1 (87.5%), *G. plecoglossi* (87.4%) and *Pleistophora finisterrensis* (86.7%) (Table 1).

MP/NJ/ML analyses of the SSU rRNA gene revealed *Glugea nagelia* sp. n. to cluster together with *Glugea* sp. from *Epinephelus awoara* (bootstrap 99%, 100%, 100%) so as to form a subclade within the clade containing all other sequenced species of *Glugea* as well as *Pleistophora finisterrensis* (bootstrap 54%, 73%, 71%) .

DISCUSSION

Members of the genus *Glugea* infect mainly teleost fish (Lom 2002) and usually display a single spore type, similar between most species and measuring about 5 µm in length. The exceptions are *Glugea fennica* Lom et Weiser, 1969 and *G. pimephales* Fantham, Porter et Richardson, 1941, whose spores measure up to 7 µm in length. This lack of precise comparative morphological features makes species differentiation on the basis of spore morphology difficult and unreliable. Nevertheless, ultrastructural comparison to other *Glugea* related species revealed that the spores of *Glugea nagelia* sp. n. display a feature only previously reported from *Glugea vincentiae* Vagelli, Paramá, Sanmartín et Leiro, 2005, i.e. the organisation of the coils of the polar filament into three rows (Vagelli et al. 2005). *Glugea vincentiae*, however, not only displays a lower number of polar filament coils (12–14), but also possesses two spore types.

Molecular-based studies show that the traditional features used for species diagnosis, namely ultrastructural features, should not be used for the discernment of microsporidian taxonomy and phylogeny (Pomport-Castillon et al. 2000, Lom and Nilsen 2003). The phylogenetic analysis presented here demonstrates that *Glugea nagelia* is closely related to a

Table 1. Comparison of some rDNA sequences: percentage of identity (top diagonal) and pairwise distance (bottom diagonal) obtained by Kimura-2 parameter analysis.

	Species	1	2	3	4	5	6	7	8	9	References
1	***Glugea nagelia*** sp. n.	-	99.5	92.5	91.7	88.7	88.1	87.5	87.4	86.7	Present study
2	*Glugea* sp.	0.005	-	92.1	91.4	91.5	90.9	91.4	91.6	91.1	Wu et al. (2005)
3	*Glugea stephani* (Hagenmüller, 1899)	0.075	0.079	-	99.5	99.7	99.1	99.5	99.6	99.3	Pomport-Castillon et al. (1998)
4	*Glugea atherinae* Berrebi and Bouix, 1978	0.083	0.086	0.005	-	99.5	99.3	99.3	99.4	99.2	Da Silva et al. (1996)
5	*Glugea hertwigi* Fantham, Porter et Richardson, 1941	0.113	0.085	0.003	0.005	-	88.2	88.9	99.2	98.8	Lovy et al. (2009)
6	*Glugea anomala* (Moniez, 1887)	0.119	0.091	0.009	0.007	0.018	-	98.0	98.1	98.0	Nilsen et al. (1998)
7	*Glugea* sp. GS1	0.125	0.086	0.005	0.007	0.011	0.020	-	99.5	98.8	Bell et al. (2001)
8	*Glugea plecoglossi* Takahashi and Egusa, 1977	0.126	0.084	0.004	0.006	0.008	0.019	0.005	-	99.0	Bell et al. (2001)
9	*Pleistophora finisterrensis* Leiro, Ortega, Iglesias, Estevez et Sanmartin, 1996	0.133	0.089	0.007	0.008	0.012	0.020	0.012	0.010	-	Nilsen et al. (1998)

Glugea sp. from the yellow grouper *Epinephelus awoara* in China, which remains unidentified to the species level, being listed in GenBank under the accession number AY090038. Similar to the host of *Glugea nagelia*, *E. awoara* also belongs to the family Serranidae, but several studies state that Microsporidia lack strict host specificity (Lom and Dyková 1992, Lom et al. 1995, Nagasawa and Cruz-Lacierda 2004, Payghan et al. 2009).

There are currently only three other microsporidians described from the Red Sea: *Pleistophora aegyptiaca*, *P. pagri* Morsy, Abdel-Ghaffar, Mehlhorn, Bashtar et Abdel-Gaber, 2012 (JF797622) and *Microsporidium aurata* (KF022044) (see Abdel-Ghaffar et al. 2012, Morsy et al. 2012, 2013). The sequences of the last two species were not included in molecular and phylogenetic analysis because they are very short, only 249 bp and 538 bp long, respectively, and they presented a low score according to BLAST homology. We can therefore conclude that *Glugea nagelia* constitutes the first record of a *Glugea* species infecting a member of the genus *Cephalopholis* Bloch et Schneider, as well as the first report of *Glugea* from the Saudi Arabian coast of the Red Sea.

Acknowledgements. We extend our appreciation to King Abdulaziz City for Science and Technology (KACST) for funding the work through the project no. ARP-32-19.

REFERENCES

Abdel-Ghaffar F., Bashtar A.R., Morsy K., Mehlhorn H., Al Quraishy S., Al-Rasheid K., Abdel-Gaber R. 2012: Morphological and molecular biological characterization of *Pleistophora aegyptiaca* sp. nov. infecting the Red Sea fish *Saurida tumbil*. Parasitol. Res. 110: 741–752.

Altschul S.F., Gish W., Miller W., Myers E.W., Lipman D.J. 1990: Basic local alignment search tool. J. Parasitol. 215: 403–410.

Bell A.S., Aoki T., Yokoyama H. 2001: Phylogenetic relationships among Microsporidia based on rDNA sequence data, with particular reference to fish-infecting *Microsporidium* Balbiani 1884 species. J. Euk. Microbiol. 48: 258–265.

Cali A., Takvorian P.M. 2003: Ultrastructure and development of *Pleistophora ronneafiei* n. sp., a microsporidium (Protista) in the skeletal muscle of an immune compromised individual. J. Eukaryot. Microbiol. 50: 77–85.

Corradi N., Keeling P.J. 2009: Microsporidia: a journey through radical taxonomical revisions. Fungal Biol. Rev. 23: 1–8.

Corradi N., Selman M. 2013: Latest progress in microsporidian genome research. J. Eukaryot. Microbiol. 60: 309–312.

Da Silva A.J., Schwartz D. A., Visvesvara G.S., De Moura H., Slemenda S.B., Pieniazek N. J. 1996: Sensitive PCR diagnosis of infections by *Enterocytozoon bieneusi* (microsporidia) using primers based on the region coding for small-subunit rRNA. J. Clin. Microbiol. 34: 986–987.

Gatehouse H.S., Malone L.A. 1998: The ribosomal RNA gene region of *Nosema apis* (Microspora): DNA sequence for small and large subunit rRNA genes and evidence of a large tandem repeat unit size. J. Invert. Pathol. 71: 97–105.

Larsson J.I.R. 1999: Identification of Microsporidia. Acta Protozool. 38: 161–197.

Lom J. 2002: A catalogue of described genera and species of microsporidian parasites in fish. Syst. Parasitol. 53: 81–99.

Lom J., Dyková I. 1992: Protozoan Parasites of Fishes. Elsevier, Amsterdam, 315 pp.

Lom J., Nilsen F. 2003: Fish microsporidia: fine structural diversity and phylogeny. Int. J. Parasitol. 33: 107–127.

Lom J., Noga E.J., Dyková I. 1995: Occurrence of a microsporean with characteristics of *Glugea anomala* in ornamental fish of the family Cyprinodontidae. Dis. Aquat. Org. 21: 239–242.

Lovy J., Kostka M., Dyková I., Arsenault G., Pecková H., Wright G.M., Speare D.J. 2009: Phylogeny and morphology of *Glugea hertwig*i from rainbow smelt *Osmerus mordax* found in Prince Edward Island, Canada. Dis. Aquat. Org. 86: 235–243.

Miwa S., Kamaishi T., Hirae T., Murase T., Nishioka T. 2011: Encephalomyelitis associated with microsporidian infection in farmed greater amberjack, *Seriola dumerili* (Risso). J. Fish Dis. 34: 901–910.

Morsy K., Abdel-Ghaffar F., Mehlhorn H., Bashtar A.R., Abdel-Gaber R. 2012: Ultrastructure and molecular phylogenetics of a new isolate of *Pleistophora pagri* sp. nov. (Microsporidia, Pleistophoridae) from *Pagrus pagrus* in Egypt. Parasitol. Res. 111: 1587–1597.

Morsy K., Bashtar A.R., Abdel-Ghaffar F., Al-Quraishy S. 2013: Morphological and phylogenetic description of a new xenoma-inducing microsporidian, *Microsporidium aurata* nov. sp., parasite of the gilthead seabream *Sparus aurata* from the Red Sea. Parasitol. Res. 112: 3905–3915.

Nagasawa K., Cruz-Lacierda E.R. 2004: Diseases of Cultured Groupers. Southeast Asian Fisheries Development Center, Aquaculture Department, Iloilo, The Philippines, 81 pp.

Nilsen F. 2000: Small subunit ribosomal DNA phylogeny of microsporidia with particular reference to genera that infect fish. J. Parasitol. 86: 128–133.

Nilsen F., Endresen C., Hordvik I. 1998: Molecular phylogeny of microsporidians with particular reference to species that infect the muscles of fish. J. Eukaryot. Microbiol. 45: 535–543.

Payghan R., Nabavi L., Jamshidi K., Akbari S. 2009: Microsporidian infection in lizard fish, *Saurida undosquamis*, of Persian Gulf. Iran. J. Vet. Res. 10: 180–185.

Pekkarinen M., Lom J., Nilsen F. 2002: *Ovipleistophora* gen. n., a new genus for *Pleistophora mirandellae*-like microsporidia. Dis. Aquat. Organ. 11: 133–42.

Pomport-Castillon C., Coste F., Romestand B., Bouix G. 1998: The *Glugea* complex in the Languedocian brackish lagoons: a multidimensional study model of fish Microsporidia. J. Eukaryot. Microbiol. 46: 15A.

Pomport-Castillon C., Joncкheere J.F., Romestand B. 2000: Ribosomal DNA sequences of *Glugea anomala*, *G. stephani*, *G. americanus* and *Spraguea lophii* (Microsporidia): phylogenetic reconstruction. Dis. Aquat. Org. 40: 125–129.

Tamura K., Dudley J., Nei M., Kumar S. 2007: MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol. Biol. Evol. 24: 1596–1599.

Tamura K., Peterson D., Peterson N., Stecher G., Nei M., Kumar S. 2011: MEGA5: Molecular Evolutionary Genetics Analysis using Maximum Likelihood, Evolutionary Distance, and Maximum Parsimony Methods. Mol. Biol. Evol. 28: 2731–2739.

Thompson J.D., Higgins D.G., Gilson T.J. 1994: Clustal W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucl. Acids Res. 22: 4673–4680.

Vagelli A., Parama A., Sanmartin M.L., Leiró J. 2005: *Glugea vincentiae* n. sp. (Microsporidia: Glugeidae) infecting the Australian marine fish *Vincentia conspersa* (Teleostei: Apogonidae). J. Parasitol. 91: 152–157.

Vossbrinck C.R., Debrunner-Vossbrinck B.A. 2005: Molecular phylogeny of the Microsporidia: ecological, ultrastructural and taxonomic considerations. Folia Parasitol. 52: 131–142.

Weiss L.M., Vossbrinck C.R. 1998: Microsporidiosis: molecular and diagnostic aspects. Adv. Parasitol. 40: 351–395.

Weiss L.M., Vossbrinck C.R. 1999: Molecular biology, molecular phylogeny, and molecular diagnostic approaches to the Microsporidia. In M. Wittner and L. Weiss (Eds.), The Microsporidia and Microsporidiosis. American Society of Microbiology, Washington, D.C., pp. 129–171.

Wu H.B., Wu Y.S., Wu Z.H. 2005: Occurrence of a new microsporidium in the abdominal cavity of *Epinephelus akaara*. Acta Hydrobiol. Sin. 29: 150–154.

Received 13 March 2014 Accepted 3 August 2014 Prepublished online 7 January 2015

Cite this article as: Abdel-Baki A.-A.S., Al-Quraishy S., Rocha S., Dkhil M.A., Casal G., Azevedo C. 2015: Ultrastructure and phylogeny of *Glugea nagelia* sp. n. (Microsporidia: Glugeidae), infecting the intestinal wall of the yellowfin hind, *Cephalopholis hemistiktos* (Actinopterygii: Serranidae), from the Red Sea. Folia Parasitol. 62: 007.