ACTA
PROTOZOOLOGICA

Acta Protozool. (2016) 55: 281–290
www.ejournals.eu/Acta-Protozoologica
doi:10.4467/16890027AP.16.028.6097

New Microsporidia, *Glugea sardinellensis* n. sp. (Microsporea, Glugeida) Found in *Sardinella aurita* Valenciennes, 1847, Collected off Tunisian coasts

Lamjed MANSOUR[1,2,3,*], Aouatef THABET[2,3], Abdel Halim HARRATH[1], Suliman Y. AL OMAR[1], Ahmed MUKHTAR[1], Shaban R. SAYED[4] and Abdel-Azeem Sh. ABDEL-BAKI[1,5]

[1] Zoology Department, College of Science, King Saud University, Saudi Arabia, P.O. Box 2455, Riyadh 11451, Saudi Arabia; [2] Unité de Recherche de Biologie intégrative et Ecologie évolutive et Fonctionnelle des Milieux Aquatiques, Département de Biologie, Faculté des Sciences de Tunis, Université de Tunis El Manar, Tunisia; [3] Faculté des Sciences de Gabes, Université de Gabès, Cité Erriadh 6072 Zrig Gabès Tunisia; [4] Transmission Electron Microscope Unit, Research Centre, College of Science, King Saud University, P.O. Box 2455, Riyadh 11451, Saudi Arabia; [5] Zoology Department, Faculty of Science, Beni-Suef University, Egypt.

Abstract. A new microsporidia *Glugea sardinellensis* n. sp. found in the teleost fish *Sardinella aurita* Valenciennes collected from the Tunisian coasts. The parasite develops in a large xenomas measuring 1–16 mm in diameter and is generally visible with naked eye in the connective tissue around the pyloric caeca of the host. Xenoma were often rounded, but would be occasionally ovoid or irregular shape, generally creamy but rarely opaque, and filled with mature spores. The spores were unikaryotic pyriform measuring 5–5.5 (5.25±0.24) µm in length and 2.5–3 (2.75±0.24) µm in width. The posterior vacuole was large and occupied more than half of the spore. Ultrastructural study indicated that the mature spore has 13–14 coils of polar filament arranged in one layer, and a rough exospore. Intermediate stages were rare and randomly distributed in the xenoma. Merogonial and sporogonial stages were uni or binucleate. The plasma membrane surrounding the meront was irregular and indented. The mean prevalence was 18.3% and it varied according to season and locality. The distribution of prevalence according to fish size indicated that small fish were primarily affected. Phylogenetic analysis using the partial sequence of the SSU rDNA showed consistent association with species of the genus *Glugea*. The most closely related species was *Glugea atherinae* Berrebi, 1979 with 98.5% similarity.

Key words: *Glugea*, fish parasites, Mediterranean Sea, phylogeny, Gulf of Gabès, round sardinella, marine.

INTRODUCTION

Microsporidia are a large and diverse group of unicellular eukaryotes that infect a wide range of hosts, from invertebrates to humans (Palenzuela *et al.* 2014, Wei *et al.* 2014). Microsporidia have been known to science for approximately 150 years, and at least 1300 to 1500 species in 187 genera have been described to date (Vávra and Lukeš 2013). Of these, approximately 20 genera have been identified in fish hosts (Stentiford *et al.* 2013). Microsporidia that cause disease or have an economic impact on aquaculture and ornamental fish occur, and some are considered a potential threat to fish populations (Palenzuela *et al.* 2014). Traditionally, the identification of microsporidians is based largely on

Address for correspondence: Lamjed Mansour, Department of Zoology, College of Science, King Saud University PO. Box: 2455, Riyadh, 11451, Saudi Arabia. Tel.: +966550145746; Fax: +966 11 4678514; E-mail address:lamjed.mansour@gmail.com

the light and ultrastructural features of the spores and/or on the characteristic cell structure of the developmental stages (Lom and Dyková 1992). Over the past two decades, utilization of rRNA sequences when describing new microsporidia is strongly suggested (Su *et al.* 2014), and Vossbrinck and Debrunner-Vossbrinck (2005) stated that the small sub-unit rRNA gene (SSUrRNA) should be used when describing basic associations among the microsporidia. Thus, most studies on microsporidia are now including both morphological and the small sub-unit rRNA gene sequence characters (Nilsen 2000, Lom and Nilsen 2003, McClymont *et al.* 2005). Round sardinella, *Sardinella aurita* Valenciennes, 1847 (Pisces, Clupeidae) is a medium-sized pelagic marine fish that is widely distributed throughout the Mediterranean and in the tropical and subtropical waters of the world (Boely 1979). Commonly, males of *Sardinella aurita* reach sexual maturity before females at a size of 155 mm for males and a size of 168 mm for females (Boely 1979, Cury Kartas 1981, Fontana 1988). Round sardinella are most common in the catches of southern Mediterranean countries, including Tunisia (Boely 1979, Kartas 1981, Cury and Fontana 1988). The catch composition of Tunisia suggested that *Sardinella aurita* is considered an abundant species, with catches totaling approximately 13300 t in 2002. Special attention is needed to investigate parasitic infections and evaluate their potential effects on populations of this important fish.

In the present study, we used both morphological and rDNA sequence characters to describe *Glugea sardinellensis* n.sp. from the connective tissue around the pyloric caeca of *Sardinella aurita* collected from the Mediterranean Sea off Tunisia.

MATERIALS AND METHODS

Fish sampling and structural characterization. During a period from 2008 and 2010 the 448 specimens of *Sardinella aurita* were collected from different localities along the Mediterranean coasts of Tunisia. Fish were caught by local fisherman and were examined for parasitic infection as they had died. Of the sampled fishes, 123 specimens were collected from Bizerte (37°16.4′N, 9°53.2′E), 204 from Tunis (36°49′5″N, 10°18′18″E) and 121 from the Gulf of Gabes (33°53′N, 10°07′E). The sizes of collected fish ranged from 11 to 19.5 cm for both sexes. Fish were grossly examined with the naked eye, as well as under a stereoscope for microsporean infection. Xenomas were isolated and the released fresh spores were observed under a light microscope. Spores were measured using an ocular micrometer and photographed with a digital camera (Leica D-LUX3). Giemsa stained spores were prepared after fixation of the smear in absolute methanol at –20°C.

For transmission electron microscopy (TEM), xenomas were fixed in 3% glutaraldehyde in cacodylate buffer 0.1 M (pH 7.4 v/v) for 4 to 8 h at 4°C. Next, samples were processed for TEM as reported elsewhere (Mansour *et al.* 2005). Briefly, after fixation of xenomas, they were washed three times and then postfixed in osmium tetroxide 1% in the same buffer. After washing with the sodium cacodylate, xenomas were dehydrated in increasing concentrations of ethanol and embedded in epoxy resin. Samples were then cut for semi-thin and ultrathin sections using a Leica Ultra-cut S ultra-microtome. Semi-thin sections (0.5–1 µm) were prepared and stained with 0.2% Azur Blue II. Ultrathin sections (60–70 nm) were stained with uranyl acetate (20 min) and lead citrate (5 min). Observations were made using a Jeol 1011 EX electron microscope (College of Science, King Saud University).

Molecular and phylogenetic analysis. For the molecular study, four cysts were extracted from different sampled fish. Freshly isolated spores were preserved in 1 X PBS as reported by Mansour *et al.* (2005). Genomic DNA extraction was performed using the Fast DNA® Kit (MP Biomedicals LLC) according to the manufacturer's instructions. The concentration and quality of genomic DNA was tested by electrophoresis in 1% gel agarose and by spectrophotometer (NanoDrop ND-1000 spectrophotometer; Thermo Fisher Scientific, Waltham, MA). Polymerase chain reactions (PCR) were carried out in a thermocycler (Techne TC 4000) apparatus using primers SF4m (5' CACCAGGTTGATYCTGCCTRD 3') (Mansour *et al.* 2005) and MICR (5′ GCGACGGGCGGTGTGTAC 3') (Mansour *et al.* 2013), amplifying a fragment of about 1245 bp of the SSU rRNA gene. The PCR were run with a final volume of 30 µl containing 50–100 ng of genomic DNA, 1 X taq polymerase buffer (Fermentas, USA), 0.2 mmol of each dNTP, 1.5 mmol of MgCl2, 0.2 pmol of each primer, and 1.5 U of Taq DNA polymerase in ultrapure (MilliQ) water. The amplification were run under the following conditions: initial denaturation for 5 min at 95°C, 30 cycles of denaturation for 30 s at 94°C, annealing for 30 s at 52°C, extension for 60 s at 72°C, and a final extension for 10 min at 72°C. The amplified DNA was visualized on 1% agarose gels stained with ethidium bromide.

Sequences of the amplified fragment were performed directly on a purified PCR using 5 µL of CEQ Dye terminator Cycle Quick Start Master Mix (Beckman Coulter) in a BioRad thermocycler apparatus using the same primes as for PCR amplification.

The consensus sequence was obtained upon both sense and anti-sense strands of samples originating from four separate PCR products. Assembly of sequences was performed using the CAP3 assembly DNA program (http://pbil.univ-lyon1.fr/cap3) (Huang and Madan 1999).

For phylogenetic analysis, similar sequences were extracted from the GenBank daTabase after a simple BLAST with the obtained sequences. Alignment of sequences was performed using CLUSTAL X v1.83 (Thompson *et al.* 1997). The obtained alignment was manually edited and ambiguous regions were identified by eye and excluded manually using BioEdit v7.0.9 (Hall 1999). A phylogenetic tree was constructed using version 5 of the MEGA software (Tamura *et al.* 2011) with the maximum likelihood (ML) and neighbour joining (NJ) methods. For maximum likelihood analyses, we used the general time reversible model, gamma distributed with invariant sites (G+I). The NJ tree was performed using the Kimura two-parameter model with gamma distribution (Kimura 1980). The genetic distance matrix was estimated using the Kimura

2-parameter method for inferring evolutionary distance in which transitions and transversions are treated separately (Kimura 1980) for 1117 positions in the final dataset. All gaps and missing data were eliminated.

RESULTS

Light microscopy. The infection appeared as spherical to ellipsoidal whitish creamy xenoma's seen by the naked eye in the connective tissue around the pyloric caeca of the host (Fig. 1A). Some xenoma were attached to the serosa of the stomach and/or were in the liver capsule (Fig. 1B). Large xenomas are mostly dark to yellowish having a very thick and hard wall that could reflect a transformation to a granulomatous cyst. The number of xenomas observed in infected fish varied from one to six, with variable size from 0.1 cm to 1.6 cm (Fig. 1B). Both xenomatous and granulomatous cyst could occur in the same fish. Granulomatous cysts were more frequent in adult fish then in juvenile. Upon squishing of xenoma, huge numbers of mature spores were released. Mature spores appear pyriform to ovoid in shape (Fig. 1C). The size of fresh spores (n = 30) was 5.25 (5–5.5) µm in length and 2.75 (2.5–3) µm in width. The posterior vacuole occupied more than the half of the volume of the spore. Histological sections of the xenoma revealed a xenoma-like structure that was filled with mature spore and some sporognic stages (Fig. 1D). The xenoma was surrounded by a thick wall and externally enveloped by thick fibroblasts and collagen fibers, trapping some mature spores (Fig. 1D and 1E). The spores and various developmental stages were randomly distributed in the xenoma.

Electron microscopy. All life cycle stages had isolated nuclei (Fig. 2). Merongonic and sporogonic stages are scarce. The presumed early uninucleate meronts and binucleate meronts were elongated and delimited by an electron dense and irregular plasma membrane, but without external reinforcement (data not shown). Sporoblasts were identified by presence of an electron-dense reinforced and serrated coat (Fig. 2A and 2B). Sporoblasts 4.8–6.8 × 2.4–3.2 become the sporogonial plasmodium, 6.5–7.2 × 2.7–3.3 µm in size, which would divide to give uninucleated sporoblasts that later differentiated to the spores (Fig. 2E). Mature spores were ovoid, typical of microsporidia, with a posterior vacuole, lamellar polaroplast, and polar tube. The spore was surrounded by an electron dense and rough exospore and a thick translucent endospore. Isofilar polar filaments generally had 13–14 turns arranged in a single layer (Fig. 2F).

Phylogeny. For this new microsporidia, 4 fragments of 650–700 bp were obtained after manual trimming. All these sequences are overlapping, giving a sequence depth of 2× to 4×. Only the first 150 bp at 5' end was sequenced one time. Alignment of the four sequenced fragments, shows only three transitions occurring one time at positions 393 (C / T), 620 (A / G) and 797 (G / A). These transition were confirmed in forward and reverse sequences. A consensus SSU rDNA sequence of 1117 bp was generated using these four fragments and deposited in GenBank under accession number KU577431. BLAST analysis revealed no identical sequence in the daTabase. The maximum of similarity was observed with *G. atherinae* Berrebi, 1979 at 98.5%, followed by *G. gasterostei* Voronin, 1974, *G. anomala* Moniez, 1887 and *G. hertwigi* Weissenberg, 1911 (Table 1). The sequence of the new species was different from *G. atherinae* by 31 nucleotides and 15 gaps through 1117 bp. The most divergent *Glugea* sequences were those of *G. jazanensis* Abdel-Baki, Tamihi, Al-Qahtani, Al-Quraishy et Mansour, 2015, *G. nagelia* Abdel-Baki, Al-Quraishy, Rocha, Dkhil, Casal et Azevedo, 2015 and *Glugea epinephelusis* Wu et Wu, 2005 (accession number, AY090038).

Phylogenetic analysis using maximum likelihood and neighbor joining methods resulted in trees with similar typologies. The new species appears within the clade grouping all *Glugea* species and closely associated with the sub-group, which contains most *Glugea* with high bootstrap support (Fig. 3).

Prevalence and seasonality. Fish sampling was conducted every month between 2008 and 2010. The overall prevalence along Tunisian coasts was approximately 18.3% (82 infected/448 total fish). This prevalence differed according to the region and increased from north to south. It was 8.1% (10/123) in Bizerte, 14.8% (30/204) in Tunis and 35% (42/121) in Gabès. The parasite was detected throughout the year in Gabes. The distribution of prevalence among different sized sardines from Gabès indicated that the smallest fish (11–12 cm) were the most affected, with prevalence near 70%. This prevalence diminished with size and was approximately 16–30% among the largest fish (Table 2). In this region, the prevalence increased from summer (10%) to spring (55%). In autumn and winter, prevalence was approximately 28% and 37% respectively. Statistical analysis revealed high significant seasonal variation of prevalence ($p < 0.001$) which is due

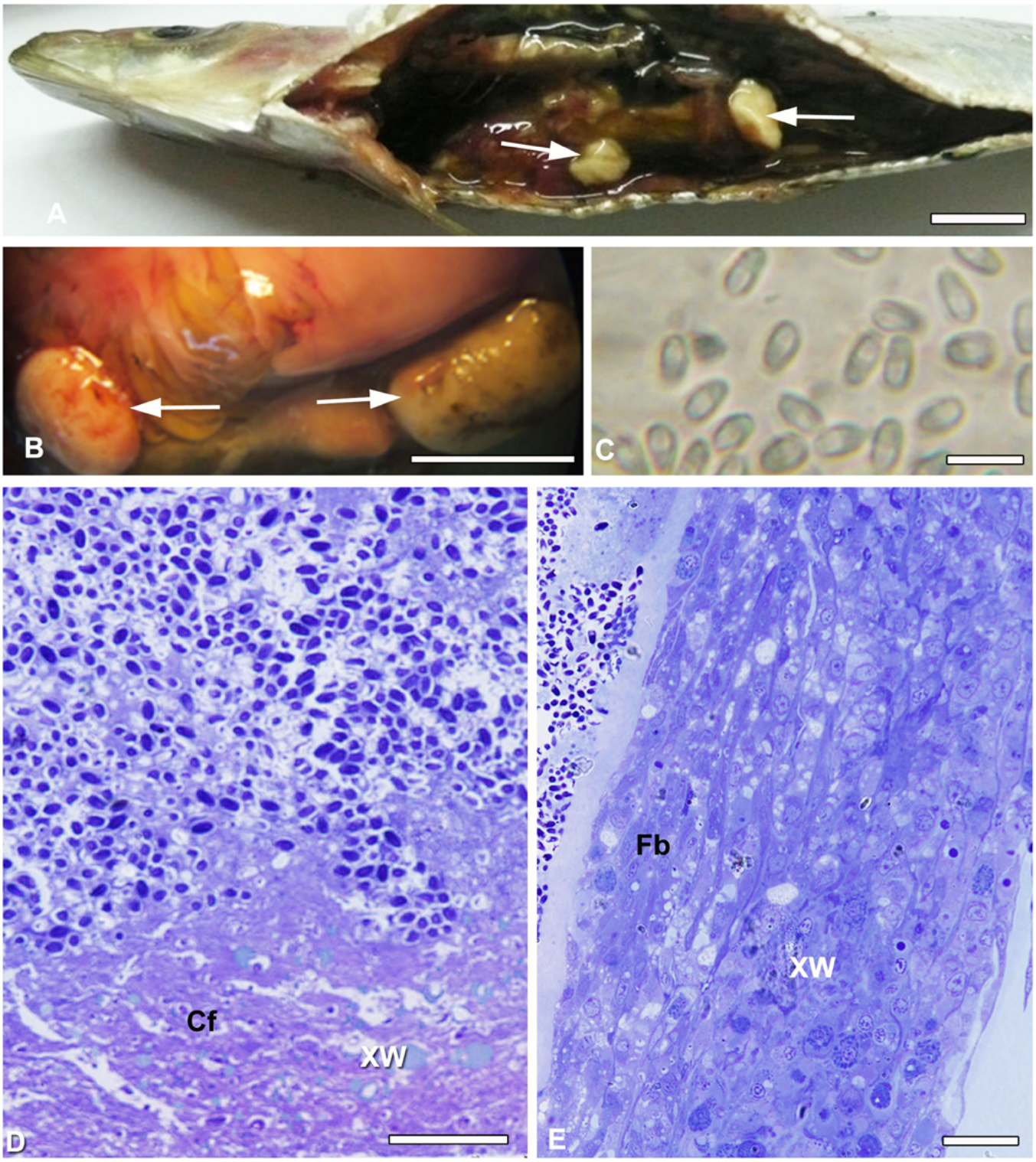

Fig. 1. *Glugea sardinellensis* n. sp. infecting *Sardinella aurita*. (A–B) Infected round sardinella showing different sized xenomas in the pyloric caeca (arrows) (scale bar: 1 cm). (C) Fresh spores of *G. sardinellensis* n. sp. (scale bar: 5 µm). (D–E) Semi-thin section of the peripheral region of the xemona showing the wall (XW) and numerous spores (scale bar: 20 µm). Cf – collagen fibers; Fb – fibroblast cells.

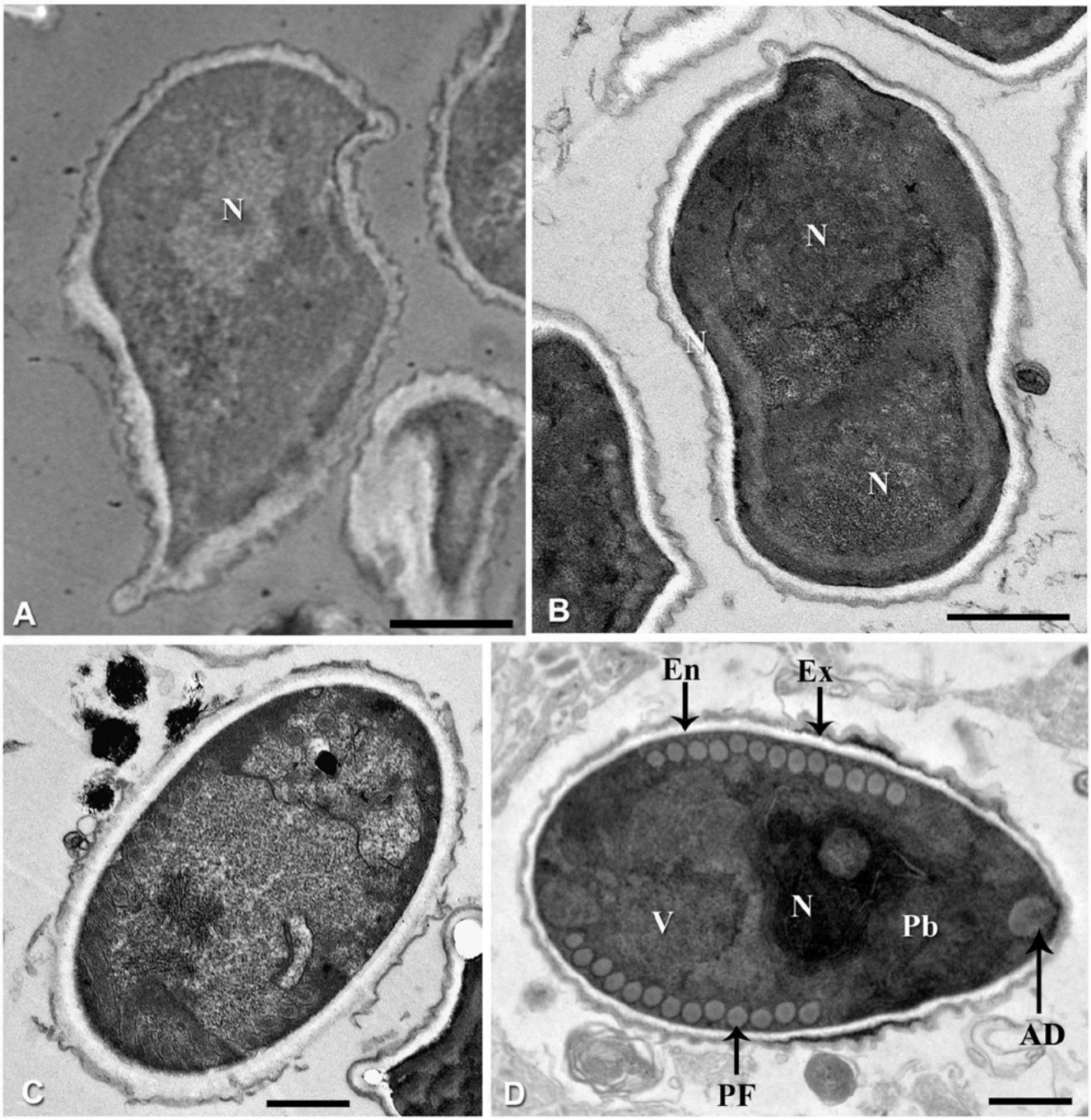

Fig. 2. Ultrastructural aspects of the developmental stages of *G. sardinellenesis* n. sp. (A) unincleated sporoblast, (B) binucleated sporoblast, (C) immature spore, (D) mature spore showing anchoring disc (AD), polar filament (PF), lamellar polaroplast (Pb), posterior vacuole (V), nucleus (N), exospore (Ex) and endospore (En). Scale bars: 1 μm.

to the highly significant difference between the prevalence of spring and the other three seasons ($p < 0.001$) and between winter and summer ($p = 0.008$). No other significant differences were recorded ($p > 0.05$). In the northern coasts of Tunisia, the parasite was detected only during autumn in Tunis and during spring in Bizerte. Small, whitish and creamy xenomas were more frequent in winter and spring. While large and dark xenomas (granulomas) were more frequent in summer and autumn.

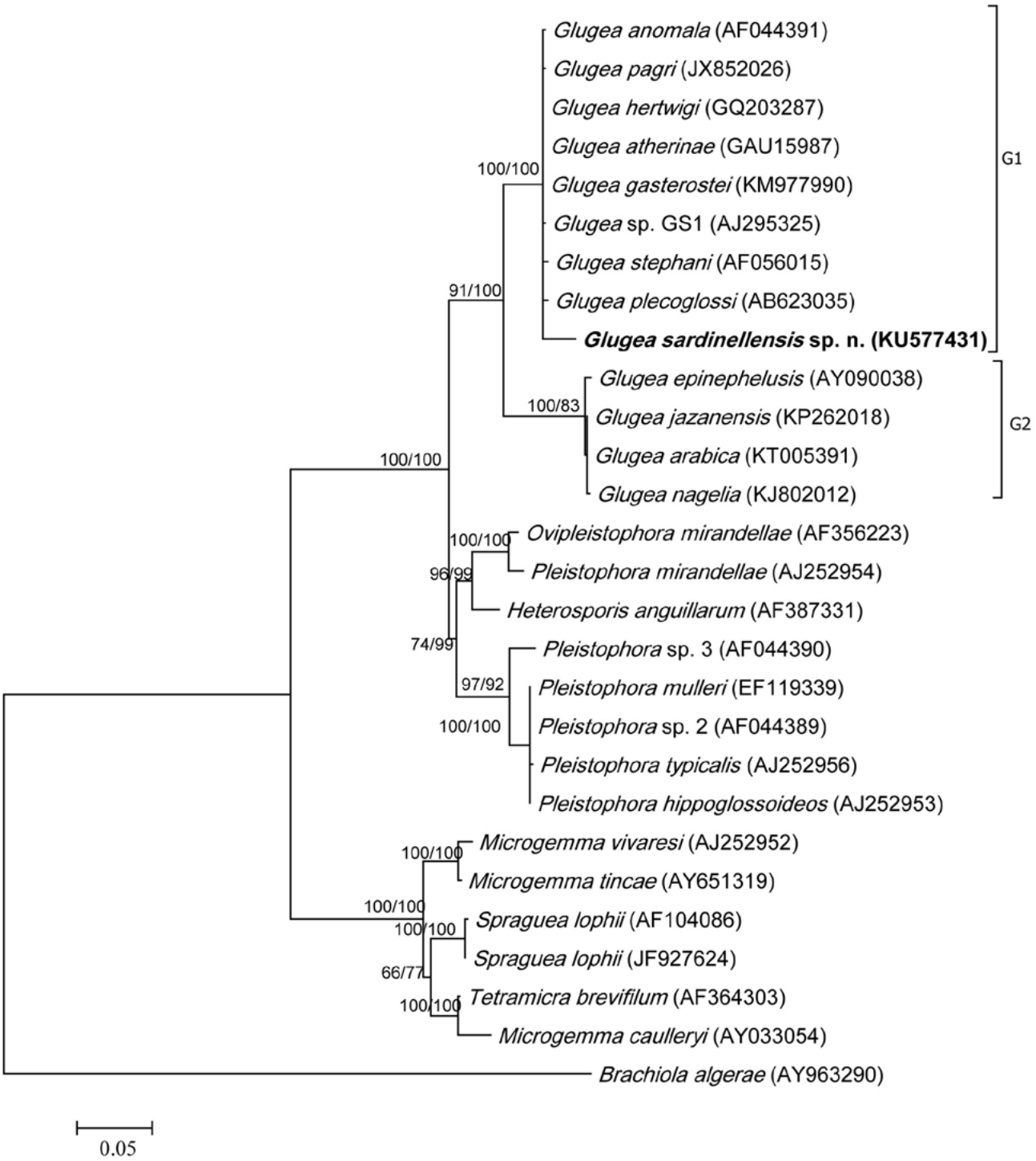

Fig. 3. Maximum likelihood phylogenetic tree based on the SSU rDNA data set selected microsporidian species showing the position of *Glugea sardinellensis* sp. n. Bootstrap supports based on 1,000 replicates from Maximum likelihood/neighbour joining analysis are indicated at each node. GenBank accession numbers for each species are reported in parenthesis. *Brachiola algerae* was used as outgroup. The scale bar shows the number of changes per site.

Table 1. Pairwise comparison of the SSU rRNA partial sequences of *Glugea* spp. including the present new species. Percentage of similarities between *G. sardinellensis* n. sp. and different *Glugea* spp. are shown in bold.

	Glugea species	Accession numbers	1	2	3	4	5	6	7	8	9	10	11	12
1	*G. sardinellensis*	KU577431												
2	*G. atherinae*	GAU15987	98.50											
3	*G. hertwigi*	GQ203287	98.12	99.95										
4	*G. gasterostei*	KM977990	98.12	99.89	99.83									
5	*G. anomala*	AF044391	97.96	99.85	99.85	99.85								
6	*G. pagri*	JX852026	97.96	99.85	99.85	99.85	99.69							
7	*Glugea* sp. GS1	AJ295325	97.96	99.85	99.85	99.85	99.69	99.69						
8	*G. plecoglossi*	AB623035	97.80	99.69	99.69	99.69	99.53	99.53	99.53					
9	*G. stephani*	AF056015	97.80	99.69	99.69	99.69	99.53	99.53	99.53	99.38				
10	*G. jazanensis*	KP262018	90.94	92.53	92.53	92.53	92.35	92.35	92.35	92.53	92.19			
11	*G. nagelia*	KJ802012	90.58	92.19	92.19	92.19	92.01	92.01	92.01	92.19	91.84	99.69		
12	*G. epinephelusis*	AY090038	90.57	92.18	92.18	92.18	92.00	92.00	92.00	92.18	91.83	99.38	99.38	
13	*G. arabica*	KT005391	90.77	92.55	92.55	92.55	92.37	92.37	92.37	92.55	92.20	99.56	99.53	99.53

Table 2. Relationship between length of examined *Sardinella aurita* and prevalence of *Glugea sardinellensis* n. sp. in the Gulf of Gabès.

Fish length (cm)	Number of examined fish* (infected)	Prevalence (%)
11–12	15 (10)	67
12–13	10 (5)	50
13–14	15 (6)	40
14–15	17 (4)	24
15–16	12 (2)	17
15–17	9 (3)	33
17–18	15 (3)	20
18–19	28 (9)	32
Total	121 (42)	34.7

Taxonomy summary

Family: Glugeidae Thélohan, 1892

Genus: *Glugea* Thélohan, 1891

Species: *Glugea sardinellensis* n. sp.

Type host: Round sardinella *Sardinella aurita* (Valenciennes) (Teleostei: Clupeidae)

Type locality: Tunisian coasts

Infection site: Connective tissue, intestine of the host

Etymology: The species name alludes to the host species

Type material: Syntypes on slide and xemona in 70% ethanol no. "Micro.-01-2016" deposited at the Museum of Zoology Department, College of Science, King Saud University, Riyadh, Saudi Arabia

DISCUSSION

The morphological and ultrastructural characteristics observed, combined with the molecular analysis of the rRNA genes, proved that the parasite described herein belongs to the genus *Glugea* Thélohan, 1891 of the phylum Microsporidia (Lom and Dyková 1992, Larsson 1999, Lom and Nilsen 2003). To date, approximately 27 *Glugea* species have been described from different geographical regions in fresh water and marine fishes (Lom and Dyková 2005, Vagelli *et al.* 2005, Su *et al.* 2014, Abdel-Baki *et al.* 2015). Of these, only *Glugea cordis* Thélohan, 1895 has been described in Family Clupeidae, the family of the host in the present study, and is found in the heart muscle of *Sardina pilchardus* (Walbaum, 1792). *G. cordis* differs in having much smaller spores (3–3.5 × 2 vs 5–5.5 × 2.5–3 µm). Regarding *Glugea* species that only infect host fish in the Mediterranean, *G. atherinae* Berrebi, 1979 has been described from the body cavity of *Atherina boyeri* Risso, 1810 from the French Mediterranean coastal lagoon. This species can be differentiated from that in the present study by slightly larger spores (4.5–6.5 × 2.6–3.3 vs 2.5–3) and a lower number of polar filament coils (9–10 vs 13–14) (see Berrebi 1979). We

examined dozens semi-thin and ultrathin sections, and intermediate stages were observed infrequently, suggesting a rapid transformation to the mature spore stage. No development in parasitophorous vesicles was observed, and all stages were identified in direct contact with the cytoplasm of the host cell. However, although development in parasitophorous vesicles has been reported among species of the genus *Glugea,* it is also common in other genera, such as *Pseudoloma*, *Loma*, and *Pleistophora* (Berrebi 1979; Lom and Pekkarinen 1999; Matthews *et al.* 2001; Azevedo and Matos 2002; Abdel-Baki *et al.* 2012, 2015).

Meronogonic and sporonogonic stages were scarce. They appeared mono- or binucleated. The binucleated meronts and sporonts were elongated and cylindrical. Merogony appears particularly surrounded by an irregular and indented plasma membrane. The size of the xenoma, its form, the surrounding envelope, and its position in the host is similar to that reported in different species of *Glugea,* including *G. atherinae* infecting *Atherina boyeri*, and *G. plecoglossi* Takahashi et Egusa 1977, *G. hertwigi* Weissenberg, 1911, *G. anomala* Moniez, 1887, *G. pimephales* Fantham, Porter et Richardson, 1941, *G. anomala*, and *G. pagri* Su, Feng, Sun, Jiang, Guoet, Xu, 2014 (Chen and Power 1972, Dyková and Lom 1977, Berrebi 1979, Dyková *et al.* 1980, Morrison *et al.* 1985, Su *et al.* 2014).

Phylogenetic analysis using a partial sequence of the SSU rRNA gene confirmed the membership of the new species to the genus *Glugea* with high bootstrap support. For the *Glugea* genus, two subclades could be distinguished; the first (G1) contained eight sequenced *Glugea* species and the second subclade (G2) contained four known species, most of them infecting fish from the Red Sea or Arabian Gulf. The species in the present study appears in the first subclade grouping the type species, *G. anomala* and the Mediterranean species, *G. atherinae.* It is noteworthy that the divergence in nucleotides among species in the same clade did not reach 0.5%. However, the differences between the species in the two groups were approximately 8%. The new species described herein exhibited the greatest differences with species in the same clade, and varies from 1.5 to 2.2%, which supports its membership to the genus *Glugea* while supporting and confirming that it is a new species.

The study of seasonal variation in parasitism revealed differences among seasons, particularly a decline in summer, and a maximum in spring, and a return to moderate levels in autumn and winter. A similar pattern has been reported for *G. atherinae* parasitizing *Atherina boyeri* in the Mediterranean coastal lagoons (Berrebi and Bouix 1980). Superposition of seasonality of both *Glugea* species could be explained by the effect of temperature, because both host fish live in similar Mediterranean environments. Similar or different patterns of seasonal variations in prevalence were reported for other microsporidia infecting either fish or invertebrate animals (Sabwa *et al.* 1984, Takvorian and Cali 1984, Khan 2004, Vinni *et al.* 2004, Abdel-Baki *et al.* 2009, Santos *et al.* 2010, Weigl *et al.* 2012, Strauss *et al.* 2013). In these studies, temperature was considered the main influencing factor for the seasonality of infection with microsporidia; however, factors related to ecobiology, behavior, and immunity of the host were not excluded. In our study, we documented the wide distribution of the parasites throughout the Tunisian coasts from the south (Gulf of Gabes) to the north (Tunis and Bizerte). However, *G. sardinellensis* is present on a year-round basis only in Gabes and it was detected only in winter in the northern coast of Tunisia (Tunis and Bizerte). Similar differential distribution was previously reported and was associated with different factors, mainly the temperature of the water, intrinsic factors of different populations of the host, hydrodynamics, and water quality (Pushkar 1979, Vethaak 1992, Barker *et al.* 1994, Kim *et al.* 1997, Kent *et al.* 1999, Kent 2000, Mansour *et al.* 2005, Leitch and Ceballos 2008, Szentgyorgyi *et al.* 2011). Regarding the relationship between fish size and prevalence, among the examined fishes, small fish (11–13 cm) were more often infected (prevalence 60–70%) than larger fish. This result corroborates previous reports related to microsporidia and other parasites. Smaller fish (juveniles) were more often infected than adults, as was the case in *Microgemma tincae* Mansour, Prensier, Jemaa, Hassine, Méténier, Vivarès et Cornillot, 2005 infecting *Symphodus tinca* (Linnaeus, 1758) from Tunisian coasts and *G. hertwigi* infecting rainbow smelt *Osmerus mordax* (Mitchill, 1814) in Lake Erie (Scarborough and Weidner 1979, Mansour *et al.* 2005). Heavily infected juvenile smelt were subject of high mortality rates in Canada and Finland (Legault and Delisle 1967, Pekcan-Hekim *et al.* 2005). Thus, for sardine in the Gulf of Gabes, the majority of juvenile fish, particularly heavily infected will likely die and will not complete their development. Only fish that are slightly infected or have strong immunity against this microsporidia will survive and continue their development. Infected adult fishes have most likely contracted the parasite only in adulthood.

CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial organization regarding the manuscript.

Acknowledgements. We extend our appreciation to the Dean of Scientific Research, King Saud University, for funding the work through the research group project number RG-1435-023.

REFERENCES

Abdel-Baki A. A., Tamihi A. F., Al-Qahtani H. A., Al-Quraishy S., Mansour L. (2015) *Glugea jazanensis* sp. nov. infecting *Lutjanus bohar* in the Red Sea: ultrastructure and phylogeny. *Dis. Aquat. Organ*. **116:** 185–190

Abdel-Baki A. S., Al-Quraishy S., Al-Qahtani H., Dkhil M. A., Azevedo C. (2012) Morphological and ultrastructural description of *Pleistophora dammami* sp. n. infecting the intestinal wall of *Saurida undosquamis* from the Arabian Gulf, Saudi Arabia. *Parasitol. Res.* **111:** 413–418

Abdel-Baki A. S., Dkhil M. A., Al-Quraishy S. (2009) Seasonality and prevalence of *Microsporidium* sp. infecting lizard fish, *Saurida undosquamis* from the Arab Gulf. *J. King Saud Univ. Sci*. **21:** 195–198

Azevedo C., Matos E. (2002) Fine structure of a new species, *Loma myrophis* (Phylum Microsporidia), parasite of the Amazonian fish *Myrophis platyrhynchus* (Teleostei, Ophichthidae). *Eur. J. Protistol*. **37:** 445–452

Barker D. E., Khan R. A., Hooper R. (1994) Bioindicators of stress in winter flounder, *Pleurowectes americanus*, captured adjacent to a pulp and paper mill in St. George's Bay, Newfoundland. *Can. J. Fish. Aquat. Sci*. **51:** 2203–2209

Ben Khaled H., Ktari N., Ghorbel-Bellaaj O., Jridi M., Lassoued I., Nasri M. (2014) Composition, functional properties and in vitro antioxidant activity of protein hydrolysates prepared from sardinelle (*Sardinella aurita*) muscle. *J. Food Sci. Technol*. **51:** 622–633

Berrebi P. (1979) Ultrastructural studies of *Glugea atherinae* n. sp., Microsporidia parasite of *Atherina boyeri* Risso 1810 (Pisces, Teleostei) in the Languedoc and Provence Lagoons. *Z. Parasitenkd*. **60:** 105–122

Berrebi P., Bouix G. (1980) *Glugea atherinae* Berrebi, 1979, Microsporidie parasite de l'Athérine *Atherina boyeri* Risso, 1810, des étangs côtiers méditerranéens. Evolution saisonnière et répartition géographique. Vie et Mileu **30:** 253–225

Boely T. (1979) Biologie de deux espèces de sardinelles (*S. aurita* Valenciennes 1847 et *S. maderensis* Lowe 1841) des côtes sénégalaises. *La Pêche Maritime* **1:** 3–7

Chakraborty K., Joseph D. (2015) Production and characterization of refined oils obtained from Indian oil sardine (*Sardinella longiceps*). *J. Agric. Food. Chem*. **63:** 998–1009

Chakraborty K., Joseph D. (2016) Concentration and sTabilization of C20-22n-3 polyunsaturated fatty acid esters from the oil of *Sardinella longiceps*. *Food Chem*. **199:** 828–837

Chen M., Power G. (1972) Infection of American smelt in Lake Ontario and Lake Erie with the microsporidian parasite Glugea hertwigi (Weissenberg). *Can. J. Zool*. **50:** 1183–1188

Cury P., Fontana A. (1988) Compétition et stratégies démographiques comparées de deux espèces de sardinelles (*Sardinella aurita* et *Sardinella maderensis*) des côtes ouest-africaines. *Aquat. Living Resour*. **1:** 165–180

Dyková I., Lom J. (1977) Tissue reaction to *Glugea anomala* infection in sticklebacks. *J. Protozool.* **24:** 50A

Dyková I., Lom J., Egusa S. (1980) Tissue reaction to *Glugea plecoglossi* infection in its natural host, *Plecoglussus altivelis*. *Folia Parasitol*. (Praha) **27:** 213–216

Hall T. A. (1999) Bioedit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucl. Acids Symp*. **41:** 95–98

Huang X., Madan A. (1999) CAP3: a DNA sequence assembly program. *Genome Res*. **9:** 868–877

Kartas F. (1981) Les clupéidés de Tunisie. Caractéristiques biométriques et biologiques. Etude comparée des populations de l'Atlantique est et de la Méditerranée. Thèse de Doctorat d'Etat, Université de Tunis, Faculté des sciences, 608

Kent M. L. (2000) Marine net pen farming leads to infections with some unusual parasites. *Int. J. Parasitol*. **30:** 321–326

Kent M. L., Dawe S. C., Speare D. J. (1999) Resistance to reinfection in chinook salmon *Oncorhynchus tshawytscha* to *Loma salmonae* (Microsporidia). *Dis. Aquat. Organ*. **37:** 205–208

Khan R. A. (2004) Effect, distribution and prevalence of *Glugea stephani* (microspora) in winter flounder (*Pleuronectes americanus*) living near two pulp and paper mills in Newfoundland. *J. Parasitol*. **90:** 229–233

Kim J. H., Ogawa K., Takahashi S., Wakabayashi H. (1997) Elevated water temperature treatment of ayu infected with *Glugea plecoglossi*: Apparent lack of involvement of antibody in the effect of the treatment. *Fish Pathol*. **32:** 199–204

Kimura M. (1980) A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. *J. Mol. Evol*. **16:** 111–120

Larsson J. I. R. (1999) Identification of microsporidia. *Acta Protozool*. **38:** 161–197

Legault R. O., Delisle C. (1967) Acute infection by *Glugea hertwigi* Weissenberg in young-of-year rainbow smelt *Osmerus Eperlanus* Mordax (Mitchill). *Can. J. Zool*. **45:** 1291–1292

Leitch G. J., Ceballos C. (2008) Effects of host temperature and gastric and duodenal environments on microsporidia spore germination and infectivity of intestinal epithelial cells. *Parasitol. Res*. **104:** 35–42

Lom J., Dyková I. (2005) Microsporidian xenomas in fish seen in wider perspective. *Folia Parasitol.* **52:** 69–81

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

Lom J., Pekkarinen M. (1999) Ultrastructural observations on *Loma acerinae* (Jirovec, 1930) comb. nov. (Phylum Microsporidia). *Acta Protozool*. **38:** 61–74

Lom J., Dyková I. (1992) Protozoan parasites of fishes. Elsevier, Amsterdam; New York

Mansour L., Ben Hassine O. K., Vivares C. P., Cornillot E. (2013) *Spraguea lophii* (Microsporidia) parasite of the teleost fish, *Lophius piscatorius* from Tunisian coasts: Evidence for an extensive chromosome length polymorphism. *Parasitol. Int*. **62:** 66–74

Mansour L., Prensier G., Jemaa S. B., Hassine O. K., Méténier G., Vivarès C. P., Cornillot E. (2005) Description of a xenoma-inducing microsporidian, *Microgemma tincae* n. sp., parasite of the teleost fish *Symphodus tinca* from Tunisian coasts. *Dis. Aquat. Organ*. **65:** 217–226

Matthews J. L., Brown A. M. V., Larison K., Bishop-Stewart J. K., Rogers P., Kent M. L. (2001) *Pseudoloma neurophilia* n. g.,

n. sp., a new microsporidium from the central nervous system of the zebrafish (*Danio rerio*). *J. Eukaryot. Microbiol*. **48:** 227–233

Elizabeth McClymont H., Dunn A. M., Terry R. S., Rollinson D., Littlewood D. T., Smith J. E. (2005) Molecular data suggest that microsporidian parasites in freshwater snails are diverse. *Int. J. Parasitol*. **35:** 1071–1078

Morrison C., Hoffman G. L., Sprague V. (1985) *Glugea pimephales* Fantham, Porter and Richardson, 1941, n. comb. (Microsporidia, Glugeidae) in the fathead minnow, *Pimephales promelas*. *Can. J. Zool*. 63:380-391

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

Palenzuela O., Redondo M. J., Cali A., Takvorian P. M., Alonso-Naveiro M., Alvarez-Pellitero P., Sitjà-Bobadilla A. (2014) A new intranuclear microsporidium, *Enterospora nucleophila* n. sp., causing an emaciative syndrome in a piscine host (*Sparus aurata*), prompts the redescription of the family Enterocytozoonidae. *Int. J. Parasitol*. **44:** 189–203

Pekcan-Hekim Z., Rahkonen R., Horppila J. (2005) Occurrence of the parasite *Glugea hertwigi* in young-of-the-year smelt in Lake Tuusulanjarvi. *J. Fish Biol.* **66:** 583–588

Pushkar E. N. (1979) Effect of temperature of the water body on the sporogony of *Thelohania fibrata* (Microsporidia) parasitic in *Odagmia Ornata* (Simuliidae). *Parazitologiya* **13:** 636–638

Sabwa D. M., Odindo M. O., Otieno W. A. (1984) Seasonal incidence of *Amblyospora* sp. (Thelohaniidae, Microsporidia) in *Culex sitiens* larvae at the Kenya Coast. *Insect Sci. Appl*. **5:** 269–272

Sonia F. O. S., Hugo D. S., Edson S. S. J., Carlos E. A., Elisângela P. S. L., Cesar A. S. T. V. C., Marco T. A. G. (2010) Environmental monitoring of opportunistic protozoa in rivers and lakes in the neotropics based on yearly monitoring. *Water Qual. Expos. Hea*. **2:** 97–104

Scarborough A., Weidner E. (1979) Field and laboratory studies of *Glugea hertwigi* (microsporida) in the rainbow smelt *Osmerus mordax*. *Biol. Bull*. **157:** 334–343

Stentiford G. D., Feist S. W., Stone D. M., Bateman K. S., Dunn A. M. (2013) Microsporidia: diverse, dynamic, and emergent pathogens in aquatic systems. *Trends Parasitol*. **29:** 567–578

Strauss U., Human H., Gauthier L., Crewe R. M., Dietemann V., Pirk C. W. W. (2013) Seasonal prevalence of pathogens and parasites in the savannah honeybee (*Apis mellifera scutellata*). *J. Invert. Pathol*. **114:** 45–52

Su Y., Feng J., Sun X., Jiang J., Guo Z., Ye L., Xu L. A. (2014) A new species of *Glugea* Thélohan, 1891 in the red sea bream *Pagrus major* (Temminck and Schlegel) (Teleostei: Sparidae) from China. *Syst. Parasitol*. **89:** 175–183

Szentgyorgyi H., Blinow A., Eremeeva N., Luzyanin S., Grzes I. M., Woyciechowski M. (2011) Bumblebees (Bombidae) along pollution gradient – heavy metal accumulation, species diversity, and *Nosema Bombi* infection level. *Pol. J. Ecol*. **59:** 599–610

Takvorian P. M., Cali A. (1984) Seasonal prevalence of the microsporidan, *Glugea stephani* (Hagenmuller), in winter flounder, *Pseudopleuronectes americanus* (Walbaum), from the New-York New-Jersey Lower Bay complex. *J. Fish Biol*. **24:** 655–663

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., Gibson T. J., Plewniak F., Jeanmougin F., Higgins D. G. (1997) The CLUSTAL-X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucleic Acids Res. **25:** 4876–4882

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

Vávra J., Lukeš J. (2013). Microsporidia and 'the art of living together'. *Adv. Parasitol*. **82:** 253–319

Vethaak A. D. (1992) Diseases of flounder (*Platichthys flesus* L) in the Dutch Wadden Sea, and their relation to stress factors. *Neth. J. Sea Res*. **29:** 257–272

Vinni M., Lappalainen J., Malinen T., Peltonen H. (2004) Seasonal bottlenecks in diet shifts and growth of smelt in a large eutrophic lake. *J. Fish Biol*. **64:** 567–579

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

Weigl S., Korner H., Petrusek A., Seda J., Wolinska J. (2012) Natural distribution and co-infection patterns of microsporidia parasites in the *Daphnia longispina* complex. *Parasitology* **139:** 870–880

Received on 17th May, 2016; revised on 19th November, 2016; accepted on 18th December, 2016