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ORIGINAL PAPER

Redefining the genus *Spraguea* based on ultrastructural and phylogenetic data from *Spraguea gastrophysus* n. sp. (Phylum Microsporidia), a parasite found in *Lophius gastrophysus* (Teleostei) from Brazil

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Abstract The ultrastructure of the fish-infecting microsporidium *Spraguea gastrophysus* found in the dorsal ganglia and kidney of the anglerfish, *Lophius gastrophysus* (family Lophiidae) collected on the Brazilian Atlantic coast is described. Each whitish xenoma (up to 3.1×1.8 mm) contains several groups of parasites. The host cells are hypertrophied and contain various parasite life stages including mature spores and several developmental stages with unpaired nuclei. Monomorphic spores are ellipsoidal, lightly curved and measure about 3.35×1.71 μm. The spore contains a gradually tapering isofilar polar filament with five to six coils arranged in a single row. The nucleus occupies a central zone of the sporoplasm where also several polyribosomes are presented. The posterior vacuole contains a voluminous spherical and granular posterosome measuring up to ~0.65 μm in diameter. The partial small subunit, intergenic spacer and partial large subunit rRNA gene were sequenced and the phylogenetic analysis places the microsporidian described here in the clade that includes all sequences of the *Spraguea* genus. The ultrastructural morphology of the xenoma and the spores of this microsporidian parasite, as well as the molecular and phylogenetic analysis, suggest the description of a new species. A redefining of the genus *Spraguea* is also done.

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Introduction

The anglerfishes of the genus *Lophius* (Family Lophiidae) have a worldwide distribution and are represented by seven species (*L. americanus*, *L. budegassa*, *L. gastrophysus*, *L. litulon*, *L. piscatorius*, *L. vaillanti* and *L. vomerinus*). Except for *L. vaillanti* and *L. vomerinus*, probably due to lack of documentation, all were found to harbour microsporidians in the nervous tissues. These parasites were first reported from the spinal ganglia of *L. piscatorius* Linnaeus, 1758 previously classified as genus *Glugea* and later identified as belonging to *G. lophii* (Doflein 1898). More detailed morphological studies developed by Mrázek (1899) reinforced that this parasite belongs to *Glugea*. However, this parasite was subsequently transferred to the genus *Nosema*, as *N. lophii* (Pace 1908), a change that was also corroborated by Weissenberg (1909, 1911a, b, c). Later, in a footnote of Weissenberg's (1976) report, it was referred to for the first time by the name "*Spraguea* n. gen." and simultaneously *Glugea lophii* was transferred to *Spraguea lophii* (Doflein 1898; Weissenberg 1976) as the type species.

The first ultrastructural data of *S. lophii*, parasite of the European anglerfish *L. budegassa* and *L. piscatorius*, both with dimorphic spores, were obtained by Loubès et al. (1979). On the other hand, the spinal and cranial ganglia of American anglerfish, *L. americanus*, caught off the northeast Atlantic coast of the USA, also indicated the presence of microsporidian spores (Takvorian and Cali 1986). Considering some ultrastructural differences in *L. americanus*, mainly having monomorphic spore type, relative to previously described dimorphic spore occurring in *L. budegassa* and *L. piscatorius* from Europe, the microsporidian was included in the genus *Glugea*, as *G. americanus* (Takvorian and Cali 1986). However, molecular results based on the SSU rRNA gene sequences suggested that this species should be transferred to the genus *Spraguea*, as *S. americana* (Lom and Nilsen 2003; Nilsen 2000; Pomport-Castillon et al. 2000). More recently, on the basis of ultrastructural and molecular studies, the xenomas found in the nervous tissues of the Japanese anglerfish *Lophius litulon* containing monomorphic spores were identified as belonging to *Spraguea americana* (Freeman et al. 2004).

There are also references to the presence of a similar microsporidian from the South American anglerfish *Lophius gastrophysus*, collected in the Brazilian and Venezuelan coasts (Jakowska 1964, 1966; Jakowska and Nigrelli 1958, 1959) but with no microscopical images or schematic drawings. This species has a high commercial value and it has been caught by the local fleets for decades. For example, landings all along the upper slope of southern Brazil (Perez et al. 2005) peaked at 7,094 tons in 2001 and 5,129 ton in 2002; however, presently only 2,500 tons are landed (Haimovici et al. 2009). Relative to fish-infecting microsporidia in Brazilian fauna, two new genera were identified: *Amazonspora* (Azevedo and Matos 2003) and *Potaspora* (Casal et al. 2008). There is also information for another four parasites: *Loma myrophis* (Azevedo and Matos 2002), *Loma psittaca* (Casal et al. 2009), *Kabatana rondoni* (Casal et al. 2010) and *Microsporidium brevirostris* (Matos and Azevedo 2004), all found in Amazonian fishes.

In the present work, we redefined the diagnosis of the genus *Spraguea* and described a new microsporidian species on the basis of the ultrastructural morphology of the spores, with special emphasis on host and tissue specificity and molecular characterization of the SSU rRNA gene.

Materials and methods

Fish, location of infection and prevalence

Thirty-six adult specimens of the marine anglerfish, *L. gastrophysus* Miranda-Ribeiro, 1915 (Teleostei, Lophiidae) (Brazilian common name "peixe-sapo pescador") (27–68 cm long; 0.550–5,600 kg weight) were collected from the Atlantic coast of "Cabo Frio" (22°50′S/42°03′W), State of Rio de Janeiro, Brazil. The fish were lightly anesthetised with MS 222 (Sandoz Laboratories), transported to the laboratory (UFF–Niterói), dissected and the infected tissues containing several whitish cysts (xenomas) were removed from the peripheral muscles of the internal abdominal cavity in contact with the dorsal nerves and kidney. The prevalence of infection was 55.5% (20 fishes in 36 examined) (11/20 females, 9/16 males) with similar rates in both sexes.

Light and electron microscopy

For light microscopy, smears of whitish xenoma and free spores were examined by a light microscope equipped with Nomarski interference-contrast (DIC) optics.

For ultrastructural studies, small fragments of the parasitized tissues containing xenomas were excised and fixed in 3% glutaraldehyde in 0.2 M sodium cacodylate buffer (pH 7.2) at 4°C for 12 h. After being rinsed overnight in the same buffer at 4°C and post-fixed in 2.0% osmium tetroxide in the same buffer for 3 h at 4°C, the fragments were dehydrated through an ascending ethanol series, followed by propylene oxide and embedded in Epon. Semithin sections were stained with methylene blue-Azur II and observed by DIC optics. Ultrathin sections were double stained with aqueous uranyl acetate and lead citrate and observed under a transmission electron microscope (TEM) JEOL 100CXII operated at 60 kV.

DNA isolation, PCR amplification and DNA sequencing

Several cysts were dissected from fishes, homogenized to isolate the spores and stored in 80% ethanol at 4°C. The genomic DNA of about 6×10^6 spores was extracted using a GenEluteTM Mammalian Genomic DNA Miniprep Kit (Sigma) following the manufacturer's instructions for animal tissue, except for the incubation time. The DNA was stored in 50 µl of TE buffer at −20°C. The majority of the region coding for the small subunit (SSU) rRNA gene was amplified by PCR using the primers V1f (5′-CACCAGGT TGATTCTGCC-3′) and 1492r (5′-GGTTACCTTGTTA CGACTT-3′) (Nilsen 2000; Vossbrinck et al. 1993). To amplify the 3′ end of the SSU, internal transcribed spacer (ITS) and 5′ end of the large subunit (LSU) rRNA gene, HG4F (5′-GCGGCTTAATTTGACTCAAC-3′) and HG4R (5′-TCTCCTTGGTCCGTGTTTCAA-3′) primers were used (Gatehouse and Malone 1998). PCR was 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 (Invitrogen products), and 3 µl of the genomic DNA. The reactions were run on a Hybaid PxE Thermocycler (Thermo Electron Corporation, Milford, MA). The amplification program consisted of 94°C denaturation for 5 min, followed by 35 cycles of 94°C for 1 min, 50°C for 1 min and 72°C for 2 min. A final elongation step was performed at 72°C for 10 min. PCR products were visualized in 5-µl aliquots with ethidium bromide staining after running on a 1% agarose gel. PCR products for the SSU gene and ITS region have approximate sizes of 1,400 and 1,100 bp, respectively. These were cleaned using the NucleoSpin Extract II (Macherey-Nagel), and then three purified PCR products were sequenced in both directions. The sequencing reactions were done using BigDye Terminator v1.1 kit (Applied Biosystems) and were run on an ABI3700 DNA analyzer (Perkin-Elmer, Applied Biosystems, Stabvida, Co., Oeiras, Portugal).

Distance and phylogenetic analysis

The various forward and reverse sequence segments were aligned manually with ClustalW (Thompson et al. 1994) in MEGA 4 software and ambiguous bases were clarified using corresponding ABI chromatograms. To evaluate the relationship of our parasite to other microsporidians, we first selected all rDNA sequences that have a fish as host (results not shown) and then we used 18 rDNA sequences, all belonging to the group 4 of the classification by Lom and Nilsen (2003). The rDNA sequence of *Nucleospora salmonis* (U781176) and *Vittaforma corneae* (L39112) were used as the outgroup. Sequences were aligned as described by Casal et al. (2008). The alignment was performed through the use of Clustal W (Thompson et al. 1994) in MEGA 4 software (Tamura et al. 2007), with an opening gap penalty of 10 and a gap extension penalty of 4 for both pairwise and multiple alignments. Subsequent phylogenetic and molecular evolutionary analyses were conducted using MEGA 4, with the sequences for microsporidian species and the outgroup species selected. Distance estimation was carried out using the Kimura-2 parameters model distance matrix for transitions and transversions. For the phylogenetic tree reconstructions, the maximum parsimony analysis was performed using the close neighbour interchange (CNI) heuristic option with a search factor of 2 and random initial trees addition of 2,000 replicates. Bootstrap values were calculated over 100 replicates.

Results

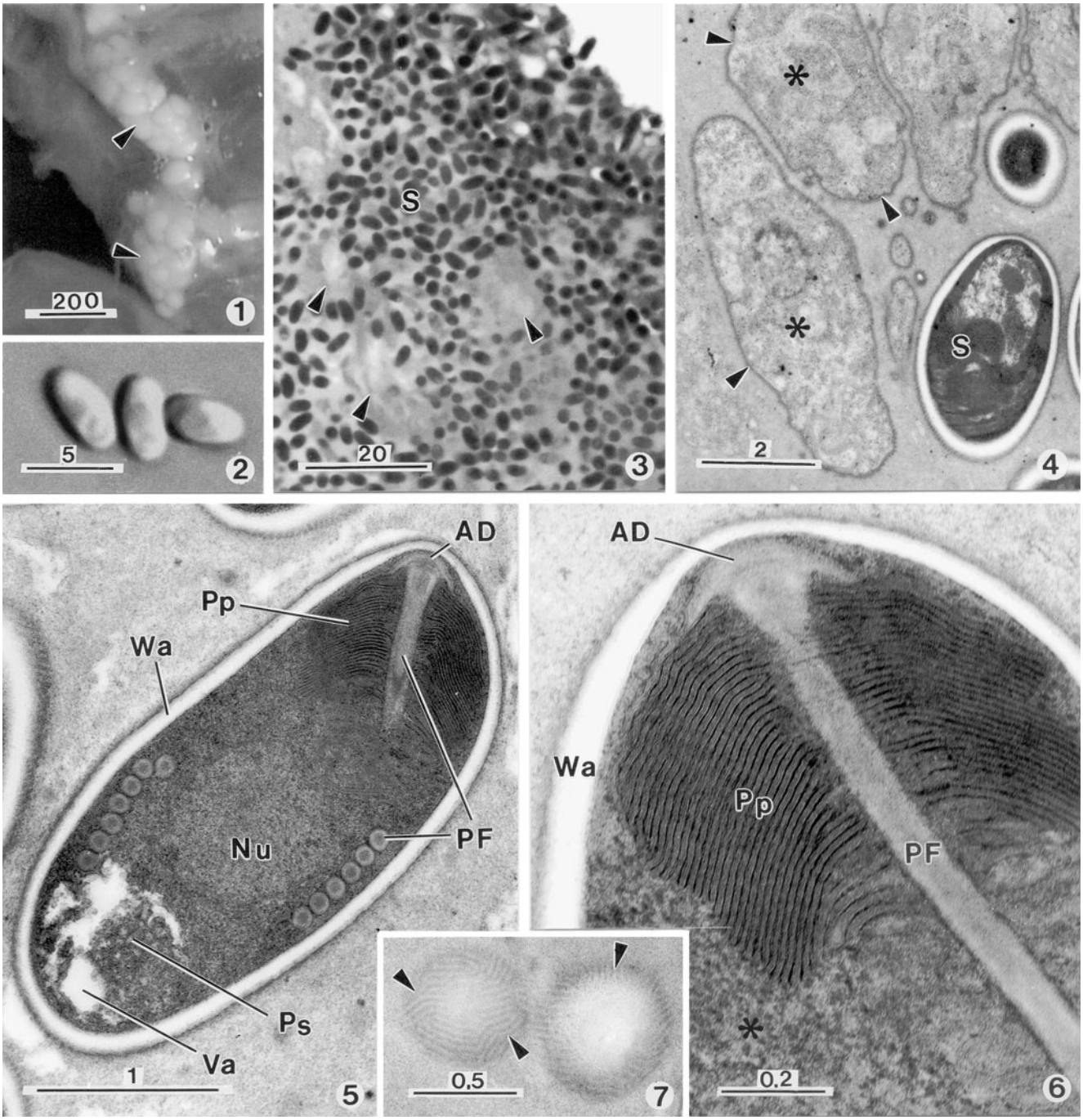
Large whitish cysts (up to 3.1×1.8 mm long) formed by several small cysts (xenomas) were macroscopically observed in the abdominal cavity in close contact with the internal abdominal muscle near the dorsal ganglia of the anglerfish, *L. gastrophysus* (Fig. 1). Similar groups of smaller cysts were observed in the kidney. After dissection and rupture of both types of xenomas, it was observed that they had numerous ellipsoidal spores (several thousand) identified as belonging to the phylum Microsporidia (Fig. 2, inset). The xenomas seen in semithin sections were irregularly shaped and contained several groups of juxtaposed cysts (Fig. 3). At high magnification, it was observed that the xenomas were formed by a wall encircling a hypertrophic cell with a central hypertrophic nucleus surrounded by numerous spores in contact with the cytoplasm of the hypertrophic host cell. Different life cycle stages of the microsporidian were observed intermingled among the spores in the matrix of the xenoma (Figs. 3 and 4).

Systematic position

Phylum Microsporidia Balbiani, 1882
Class Marinosporidia Vossbrinck and Debrunner-Vossbrinck, 2005
Family Spraguidae Weissenberg, 1976
Genus *Spraguea* Weissenberg, 1976
Species *Spraguea gastrophysus* n. sp.

Description of the species

Name: *Spraguea gastrophysus* n. sp.
Type host: *Lophius gastrophysus* Miranda-Ribeiro, 1915 (Teleostei, Lophiidae).
Type locality: Atlantic coast of Cabo Frio (22°50′S/42°03′ W), State of Rio de Janeiro, Brazil.



Figs. 1–7 Light and electron micrographs of the xenomas, developmental stages and spores of *Spraguea gastrophysus* n. sp., a parasite of the peripheral muscle of the internal abdominal cavity of the teleost *Lophius gastrophysus* (*Scale bars* in μm). **1**. Some grouped xenomas (*arrowheads*) were observed in DIC. **2**. Fresh spores observed in DIC. **3**. Semithin section of the periphery of a xenoma showing numerous spores (*S*) and other developmental stages (*arrowheads*). **4**. A group of sporoblasts (*) showing an electron dense wall (*arrowheads*) and spores (*S*). **5**. A mature spore longitudinally sectioned showing the wall (*Wa*), anchoring disc (*AD*), polaroplast (*Pp*), polar filament (*PF*), nucleus (*Nu*), posterosome (*Ps*) and posterior vacuole (*Va*). **6**. Ultrastructural details of the anterior portion of a mature spore showing the polaroplast organization (*Pp*), spore wall (*Wa*), anchoring disc (*AD*), polar filament (manubrium) (*PF*) and numerous ribosomes (*). **7**. Tangential section of the spore surface showing the external ornamentation seeming a fingerprint-like structure (*arrowheads*)

Location in the host: Xenoma in the dorsal muscle of the internal abdominal cavity and kidney.

Prevalence of infection: Twenty of 36 examined (55.5%) (11/20 females, 9/16 males) with similar rates in both sexes.

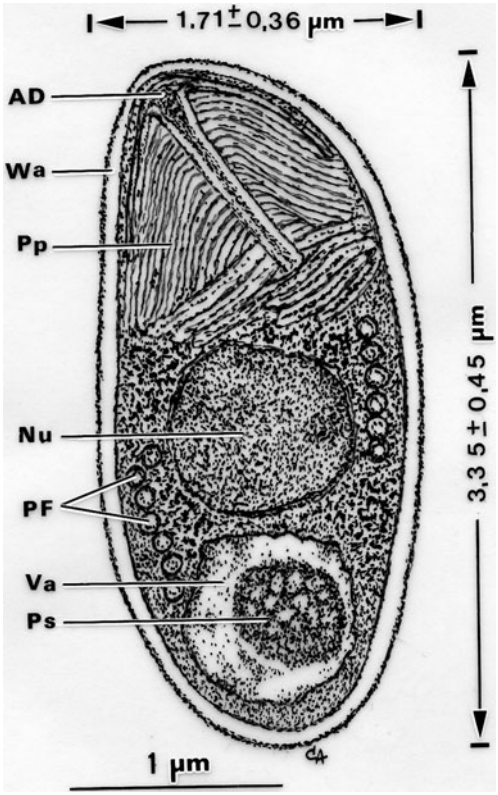


Fig. 8 Schematic drawing of a spore of *Spraguea gastrophysus* n. sp., showing all typical specific structures of the microsporidian spore, such as anchoring disc (*AD*), nucleus (*Nu*), polar filament (*PF*), polaroplast (*Pp*), vacuole (*Va*), posterosome (*Ps*) and wall (*Wa*)

Type specimens: One glass slide with a semithin section of a xenoma containing different developmental stages, mainly mature spores of hapantotype were deposited in the Microsporidia Type Slide Collection at the "Instituto Nacional de Pesquisa da Amazônia" (INPA), Manaus, Brazil, under acquisition number (001/11).

Etymology: The specific epithet "gastrophysus" is derived from the specific epithet of the host species.

Description of the spores

Ellipsoidal spores measuring 3.35±0.45×1.71±0.36 µm (n=50) (Fig. 2), and containing all the typical characteristics of the Microsporidia (Fig. 5) were observed in the two types of xenomas. The spore wall was 75.3±2.9 (n=20) nm thick and consisted of a thin electron-dense exospore 18.2±2.2 (n=20) nm and a thick electron-lucent endospore 60.6±3.8 (n=20) nm thick (Fig. 6). The spore wall was thinnest (~45 nm thick) over the sub-apical positioned anchoring disc.

The external surface of the exospore is ornamented with numerous fingerprint-like structures uniformly distributed at the periphery of the spore wall (Fig. 7). The anchoring disc was in close contact with the internal apical portion of the spore wall (Figs. 5 and 6). The anchoring disc was located in the apical region of the spore in an eccentric position in relation to the spore axis, in continuity with the anterior part of the polar filament (PF) (manubrium) (Figs. 5 and 6). The anterior part of the PF measured 120.2±5.1 nm (n=20) and was passing through the polaroplast with an angle of tilt of ~30°. The PF was isofilar, measuring 100–110 nm in diameter, arranged into five to six coils in one row (Fig. 5). The polaroplast consisted of a complex membranous system with two distinct kinds of lamellae. The anterior group of lamellae was closely packed with parallel lamellae (~12 nm between the folds) and the posterior group was packed with widely spaced and irregularly organized lamellae (Fig. 6). The spores were monomorphic and uninucleate and the nucleus occupied a position between the apical polaroplast and the basal vacuole (Fig. 5). The posterior vacuole occupied about one-quarter of the total volume of the spore (Fig. 5) and contained a spherical electron dense posterosome, measuring about 0.65 nm. Diagrammatic illustration of spore, based on DIC and TEM observations, is present in Fig. 8

Molecular and phylogenetic analysis

In total, 1,824 bp sequence (GC content 46.8%) representing the partial SSU, complete ITS and partial LSU rDNA of the parasite was successfully amplified and deposited in GenBank with the accession number (GQ868443). A Blast search of the GenBank database with the sequence obtained from *S. gastrophysus* n. sp. detected close matches to other microsporidian rRNA sequences, namely, with all *Spraguea* spp. sequences. All microsporidian sequences that have a fish as host were aligned and the most parsimonious tree showed *S. gastrophysus* n. sp. grouped with all *Spraguea* spp. A second alignment with only sequences from the group 4 designed by Lom and Nilsen (2003) was performed. The 5′ end and 3′ end SSU rDNA were trimmed, resulting in an alignment with 1,454 bp. Before the phylogenetic analysis was performed only those sites which could be unambiguously aligned among all microsporidian and the outgroup taxa were used, resulting in an alignment 1,317 bases long (Fig. 9).

The Kimura-2-parameter model pairwise distance results showed that sequences with the greatest affinity belonged to genus *Spraguea*. Percent similarities were 99.4% (*S. lophii* AF104086, *S. lophii* AF033197, *S. americana* AY465876) and 99.1% (*S. lophii* AF056013, *S. americana* AF056014) (Table 1). The final phylogenetic trees were constructed using the maximum parsimony (MP) showed that all *Spraguea* spp. formed a monophyletic group with 100% bootstrap support. A bootstrap support of 68% was found for the clade composed of all *Spraguea* spp., *Microgemma* spp. and the monotypic genus *Tetramicra*. Upon analysis of the sequences, a small number of gaps, insertions, transitions and transversions were found (Table 2).

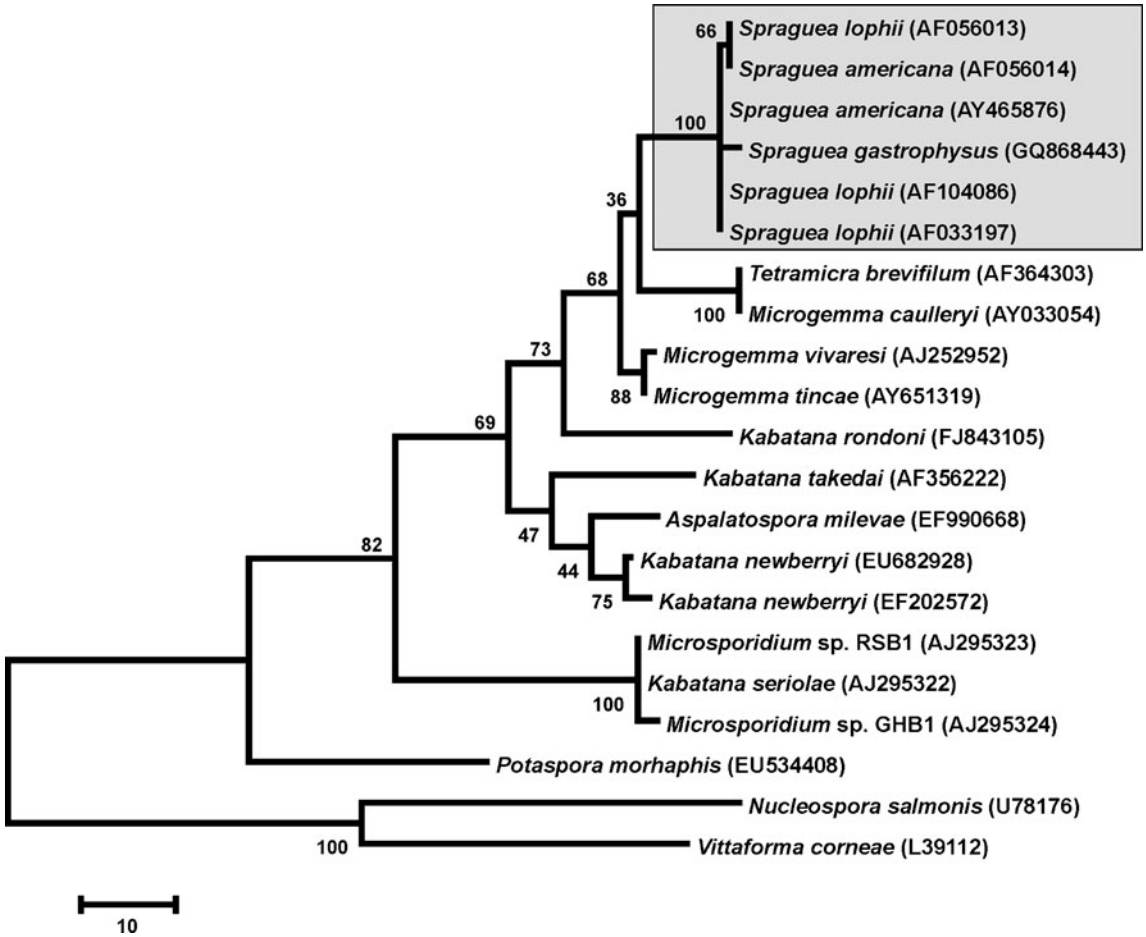


Fig. 9 The maximum parsimony tree of SSU rDNA sequences of *Spraguea gastrophysus* n. sp. and the microsporidian species clustered in group 4. The numbers on the branches are bootstrap confidence levels on 100 replicates. GeneBank accession numbers are in *parentheses* after the species names and the scale is given under the tree

Discussion

The parasite described in this paper presents the typical morphology and characteristics of the phylum Microsporidia (Lom and Dyková 1992; Cali and Takvorian 1999; Larsson 1999). Among the 18 genera infecting fish, 12 of these produce xenomas: *Amazonspora* Azevedo and Matos 2003; *Glugea* Thélohan, 1891; *Ichthyosporidium* Caullery and Mesnil, 1905; *Loma* Morrison and Sprague, 1981; *Microfilum* Faye, Toguebaye and Bouix, 1991; *Microgemma* Ralphs and Matthews, 1986; *Myosporidium* Baquero, Rubio, Moura, Pieniazek and Jordana, 2005; *Neonosemoides* Faye, Toguebaye and Bouix, 1996; *Pseudoloma* Matthews, Brown, Larison, Bishop-Stewart, Rogers and Kent, 2001; *Potaspora* Casal, Matos, Teles-Grilo and Azevedo 2008; *Spraguea* Weissenberg 1976 and *Tetramicra* Matthews and Matthews 1980.

Among these microsporidians, the genus *Spraguea* is a typical case of close relationship between the kind of parasite, host and the place of infection. Presently, it is known that five (*L. piscatorius*, *L. budegassa*, *L. americanus*, *L. litulon and L. gastrophysus*) of seven lophiid species are parasitized in the nervous tissues (spinal nerves of the vertebral column, trigeminal nerves, vagal nerves or in the medulla oblongata region of the hind brain) by microsporidia belonging to the genus *Spraguea* (Jakowska 1964; Takvorian and Cali 1986; Weissenberg 1911c, 1976). One exception for this was observed in the anglerfish *Lophius budegassa* in Spain for the reason that the microsporidians *Tetramicra brevifilum* and *Spraguea lophii* were simultaneously found in hepatocytes and musculature, respectively (Maíllo et al. 1998). The species *Tetramicra brevifilum* has no ornamentation on the exospore, is phylogenetically close to the *Spraguea* spp. and frequently parasitizes the connective tissues of the musculature of *Scophtalmus maximus* (Matthews and Matthews 1980).

Irrefutably, the parasite described in this study is morphologically and phylogenetically close to two *Spraguea* species previously reported in different anglerfish species. *Spraguea lophii* was described in *Lophius piscatorius* and *L. budegassa* on the European Atlantic and Mediterranean

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

	1	2	3	4	5	6	7	8	9	10
(1) *Spraguea gastrophysus* n. sp.		99.4	99.4	99.4	99.1	99.1	95.8	95.5	93.6	93.6
(2) *Spraguea lophii* AF104086	0.006		100	100	99.7	99.7	96.4	96.1	94.2	94.2
(3) *Spraguea lophii* AF033197	0.006	0.000		100	99.7	99.7	96.4	96.1	94.2	94.2
(4) *Spraguea americana* AY465876	0.006	0.000	0.000		99.7	99.7	96.4	96.1	94.2	94.2
(5) *Spraguea lophii* AF056013	0.009	0.003	0.003	0.003		100	96.1	95.8	93.9	93.9
(6) *Spraguea americana* AF056014	0.009	0.003	0.003	0.003	0.000		96.1	95.8	93.9	93.9
(7) *Microgemma tincae* AY651319	0.042	0.036	0.036	0.036	0.039	0.039		99.7	96.1	96.1
(8) *Microgemma vivaresi* AJ252952	0.045	0.039	0.039	0.039	0.042	0.042	0.003		95.8	95.8
(9) *Microgemma caulleryi* AY033054	0.064	0.058	0.058	0.058	0.061	0.061	0.039	0.042		100
(10) *Tetramicra brevifilum* AF364303	0.064	0.058	0.058	0.058	0.061	0.061	0.039	0.042	0.000	

coasts (Loubès et al. 1979). *Spraguea americana*, first described as *Glugea americanus*, was described on the US Atlantic coast (in *L. americanus*) (Takvorian and Cali 1986) and later in *L. litulon* from the Japanese coast (Freeman et al. 2004). The molecular data has shown that the *Glugea americanus* sequences are close to all other *Spraguea lophii* sequences and consequently it was transferred to the genus *Spraguea* and renamed *S. americana* (Nilsen 2000; Pomport-Castillon et al. 2000; Lom and Nilsen 2003).

The morphology of the spores shows several similarities when compared with other uninuclear spores of the *Spraguea* genus, except for the thickness of the two layers of the wall (exospore and endospore). The spore wall found in *L. gastrophysus* is thicker than that of other species (Table 3).

For some genera, such as *Amazonspora* (Azevedo and Matos 2003), *Kabatana* (Lom et al. 1999, 2001; McGourty et al. 2007) and *Spraguea* (Loubès et al. 1979; Takvorian and Cali 1986; Freeman et al. 2004) the fingerprint-like structures of the external surface of the exospore wall are a morphological characteristic common at all species. Usually, the external ornamentation is regularly distributed and the elevations on the surface of the mature spores, when they are observed in tangential section, present a hexagonal fingerprint-like shape (genus *Amazonspora*) or a tubular shape (genera *Kabatana* and *Spraguea*). As in *S. lophii* and *S. americana* this ultrastructural detail was also observed on the surface of *S. gastrophysus* n. sp. Inside the posterior vacuole, there is a spherical electron dense structure, similar to that reported in *S. americana* caught in the American North Atlantic coast (Takvorian and Cali 1986) and from Japanese lophiid species (Freeman et al. 2004).

The most parsimonious phylogenetic tree (Fig. 9) clustered all *Spraguea* sequences in the same clade (bootstrap 100%) and this cladogram had a similar topology to previous described trees (Lom and Nilsen 2003; Freeman et al. 2004; Casal et al. 2008). Comparison of the SSU rDNA sequence of *S. gastrophysus* n. sp. with all the other known sequences from *Spraguea* infections from Europe, Japan and America showed that the genetic distances range from 0.6% to 0.9% (Table 1). The genetic distance between all previously reported *Spraguea* infections is less than or equal to 0.3%, a result which is in accordance with that obtained by Freeman et al. (2004). It suggests that the microsporidia found in the anglerfish *L. gastrophysus* from South America is the most phylogenetically distanced of the three *Spraguea* species. Small genetic distances using the sequence of the SSU rDNA have been reported in other microsporidian species. Interestingly, in our analysis, the pairwise sequence variation between *Microgemma tincae* and *M. vivaresi* (0.3% instead 1%) was lower than that observed by Mansour et al. (2005). The similar situation occurs between two

Table 2 Comparative analysis of all *Spraguea* spp. sequences with the obtained in this study from of *Lophius gastrophysus*

The SSU rDNA length, the number the gaps, insertions, transitions and transversions are included

Spraguea spp.	Length of SSU used in the analysis	Gaps	Insertions	Transitions	Transversions
S. lophii (1) AF104086	1287	7	5	9	4
S. lophii (2) AF033197	1319	0	12	7	7
S. lophii (3) AF056013	1175	0	9	7	6
S. americana (1) AF056014	1174	2	10	5	6
S. americana (2) AY465876	1206	0	4	2	1

Table 3 Comparative measurements from *Spraguea* spp.

Parasite *Spraguea* spp.	Host species	Country (region)	Spore dimorphism	Spore dimensions (in µm)	SSO	Wall thick (in nm) Exospore/Endospore	PFC	References
S. lophii	*L. piscatorius*	France (Atlantic coast)	+	3.5×1.5 (*) 4.0×1.25 (**)	+	?	5–6 (*) 3–4 (**)	Loubès et al. 1979
S. lophii	*L. budegassa*	France (Mediterranean)	+	3.5×1.5 (*) 4.0×1.25 (**)	–	?	5–6 (*) 3–4 (**)	Loubès et al. 1979
S. americana	*L. americanus*	USA (Atlantic coast)	–	2.8×1.5	+	12.5/70	6–9	Takvorian and Cali 1986
S. americana	*L. litulon*	Japan	–	3.4×1.8	+	~30/~65	5–8	Freeman et al. 2004
S. gastrophysus n. sp.	*L. gastrophysus*	Brasil (Atlantic coast)	–	3.35×1.71	+	~18/~60	5–6	Present study

All species parasite a teleost fish from genus *Lophius*

SSO spore surface ornamentation, *PFC* polar filament coils; spore of monokaryon type (*), spore of diplokaryon type (**), positive (+), negative (–), no data (?)

species, *Tetramicra brevifilum* and *M. vivaresi*, belonging to different genera. According to Cheney et al. (2001), the genetic distance between *Pleistophora hippoglossoideos* from the flatfish *Hippoglossoides platessoides* and *Pleistophora typicalis* from the sea scorpion fish *Myoxocephalus scorpius* is only 0.4%. Presently, it is known that the SSU rDNA sequence for fish-infecting microsporidia does not provide sufficient variation to fully resolve the phylogeny between closely related species, even including the ITS and a part of the LSU rDNA (Lom and Nilsen 2003). Unfortunately for the previously described *Spraguea* species there is no genetic information for these regions of the rDNA.

According to the original description by Loubès et al. (1979), the genus *Spraguea* is a dimorphic microsporidian that produces two types of spores in two distinct developmental sequences. One sequence is characterized by all stages having unpaired nuclei and polysporoblastic sporogony that produce uninucleate spores whereas the other sequence is characterized by diplokarya and disporoblastic sporogonic stages that give rise to slender curved diplokaryotic spores. Curiously, the spore dimorphism of the genus and species type is in contradiction with all other ultrastructural descriptions. Apparently, this is an exclusive characteristic of the *Spraguea* infections from *Lophius piscatorius* and *L. budegassa* from European species because the infections from the American and Japanese species only produce uninucleate spores. Definitely, as recommended by Lom (2002), Lom and Nilsen (2003) and Freeman et al. (2004), the genus diagnosis needs to be redefined since the exceptions must not be general characteristics of the genus.

Diagnosis of genus *Spraguea*

Monophyletic group of parasite that infect ganglion cells of the Lophiid species. Xenoma bounded by a single cell membrane and with a single hypertrophic nucleus. Monomorphic or dimorphic development sequences. Always present is one sequence characterized by isolated nucleus in all stages and polysporoblastic sporogony where uninucleate spores form by radial cleaving of the sporogonial plasmodium. Sometimes another sequence also develops where all stages present diplokarya and by disporoblastic sporogony produces curved spores.

Considering the morphological and molecular data, we redefined the genus *Spraguea* and designated a new species that should be included in this genus with the name *S. gastrophysus* n. sp.

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