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FISH PARASITOLOGY - REVIEW

Pseudokabatana alburnus n. gen. n. sp., (Microsporidia) from the liver of topmouth culter *Culter alburnus* (Actinopterygii, Cyprinidae) from China

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Abstract

We describe the type species of a novel genus of microsporidian parasite, *Pseudokabatana alburnus* n. gen. n. sp., infecting the liver of topmouth culter, *Culter alburnus* Basilewsky, 1855, from Lake Poyang off Xingzi county, Jiangxi Province, China. The parasite elicits formation of spherical xenomas of up to 1.2 mm in diameter containing all observed life stages from early merogonal plasmodia to mature spores contained within the cytoplasm of host hepatocytes. Merogonal plasmodia existed in direct contact with the host cytoplasm and contained up to 20 visible nuclei. Plasmotomy of the multinucleate plasmodium led to formation of uninucleate cells in which the nucleus underwent further division to form bi-nucleate presporonts, sporonts (defined by cells with a thickened endospore) and eventually sporoblasts (containing pre-cursors of the spore extrusion apparatus). Mature spores were pyriform and monokaryotic, measuring 2.3 ± 0.19 μm long and 1.3 ± 0.10 μm wide. Spores possessed a bipartite polaroplast and 5–6 coils of a polar filament, in a single rank. The obtained partial SSU rRNA gene sequence, 1383 bp in length, did not match any of microsporidia available in GenBank. SSU rDNA-based phylogenetic analysis indicated a new taxon branching with *Kabatana rondoni*, a parasite infecting the skeletal muscle of *Gymnorhamphichthys rondoni* from the Amazon River. Due to different host and tissue tropism, the novel taxon did not fit the diagnostic criteria for the genus *Kabatana.* Further, based on SSU rDNA-inferred phylogenetic analyses, different ultrastructural features of developmental stages, and ecological considerations, a new genus *Pseudokabatana* and type species *Pseudokabatana alburnus* n. sp. was erected for the parasite in topmouth culter.

Keywords *Kabatana* · *Pseudokabatana alburnus* n. gen. n. sp. · Ultrastructure · Xenoma

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Introduction

Microsporidia are ubiquitous obligate intracellular parasites that can infect diverse invertebrate (frequently arthropods) and vertebrate (including fish) hosts (Simakova et al. 2018; Abdel-Ghaffar et al. 2012; Casal et al. 2016; Stentiford et al. 2013a, 2015). At least 1600 species belonging to over 200 recognized genera have been reported worldwide, and of them, more than 160 species in 21 genera are known to infect diverse ornamental, marine, and freshwater fishes from wide-ranging niches (Lom and Nilsen 2003; Kent et al. 2014; Stentiford et al. 2013a). Among fish-infecting microsporidia, most are described from bony fish, some of which are known to cause important health issue in aquaculture (Kent et al. 2014).

Despite the presence of diverse wild and cultured fish populations, few studies on this important parasite group have been conducted in China. In the last century, several species of *Glugea* and *Pleistophora* have been recorded from fish

hosts in China (including *Mylopharyngodon piceus*, *Ctenopharyngodon idellus*, *Hypophthalmichthys molitrix*, *Aristichthys nobilis*, *Cyprinus carpio*, *Carassius auratus*, *Xenocypris argentea*, *Tilapia nilotica*, *Ophiocephalus maculatus*, *O. argus*, and *Pseudorabora parva*) (Chen 1955; Chen 1956a, b; Chen and Xie 1960; He and Li 1985). Further, *Agmasoma penaei* was described infecting several penaeid shrimp hosts by He (1988) based solely on light microscopic observations. Since then, only sporadic reports of aquatic microsporidian infections have been made. More recently, several commercially important microsporidians have been described from intensive aquatic animal culture, including enteric microsporidiosis of groupers (Xu et al. 2017, Yan et al. 2018), slow growth syndrome in penaeids associated with *Enterocytozoon hepatopanaei* (Tourtip et al. 2009; Aranguren et al. 2017), and so-called toothpaste disease of swimming crab (Wang et al. 2017). Finally, several fish-infecting species of *Glugea* and *Microsporidium* were ultrastructurally and molecularly characterized from hosts collected from wild marine hosts (Wu et al. 2005; Su et al. 2014). However, the diversity of aquatic microsporidian in China is undoubtedly underestimated, especially within freshwater habitats.

Lake Poyang, in the lower reaches of Yangtze River, is the largest freshwater lake in China at 4125 km^2. To date, 134 fish species have been recorded from the lake, around one third of the amount of fish species in the Yangtze River basin (Cao 2011; Fang et al. 2016). To the best of our knowledge, no microsporidian parasite has previously been described from hosts collected from this natural watershed. As part of ongoing survey work exploring diversity in freshwater microsporidia from China, we analyzed specimens of the topmouth culter (*Cluter alburnus*) collected from Lake Poyang in 2016. We used morphological, ultrastructural, and molecular data to characterize a novel microsporidian parasite infecting the liver of *C. alburnus*. A new taxon *Pseudokabatana alburnus* n. gen. n. sp. was erected by comparing the *C. alburnus* parasite to most closely related microsporidian genera (including *Kabatana*) within Clade 5 of the Microsporidia.

Materials and methods

Sample collections

Eight specimens of topmouth culter (*C. alburnus*) ranging from 23.0 g to 55.4 g in body weight were captured by gill nets from Lake Poyang off Xingzi county, Jiangxi province, China (29°27′3″N, 116°01′32″E) during July 2016. Fish were transported to a local fish diseases laboratory for parasitological examination and dissection. After visual examination of the body surface, fish were anesthetized by an overdose of MS 222 (Sigma, Germany) and necropsy was conducted. Presumptive microsporidian xenomas were dissected from the surface of the liver using a dissection microscope (Olympus SZ51) prior to wet mounting on clean glass slides and examination using light microscopy Motic BA210 (Motic, China). Images were obtained using Olympus BX 53 microscope equipped with an Olympus DP72 digital camera (Olympus, Japan). Other xenomas were dissected from the surface of the liver and preserved in 95% ethanol and 2.5% glutaraldehyde in 0.1 M sodium cacodylate buffer (PH 7.4) for molecular analyses and electron microscopy, respectively.

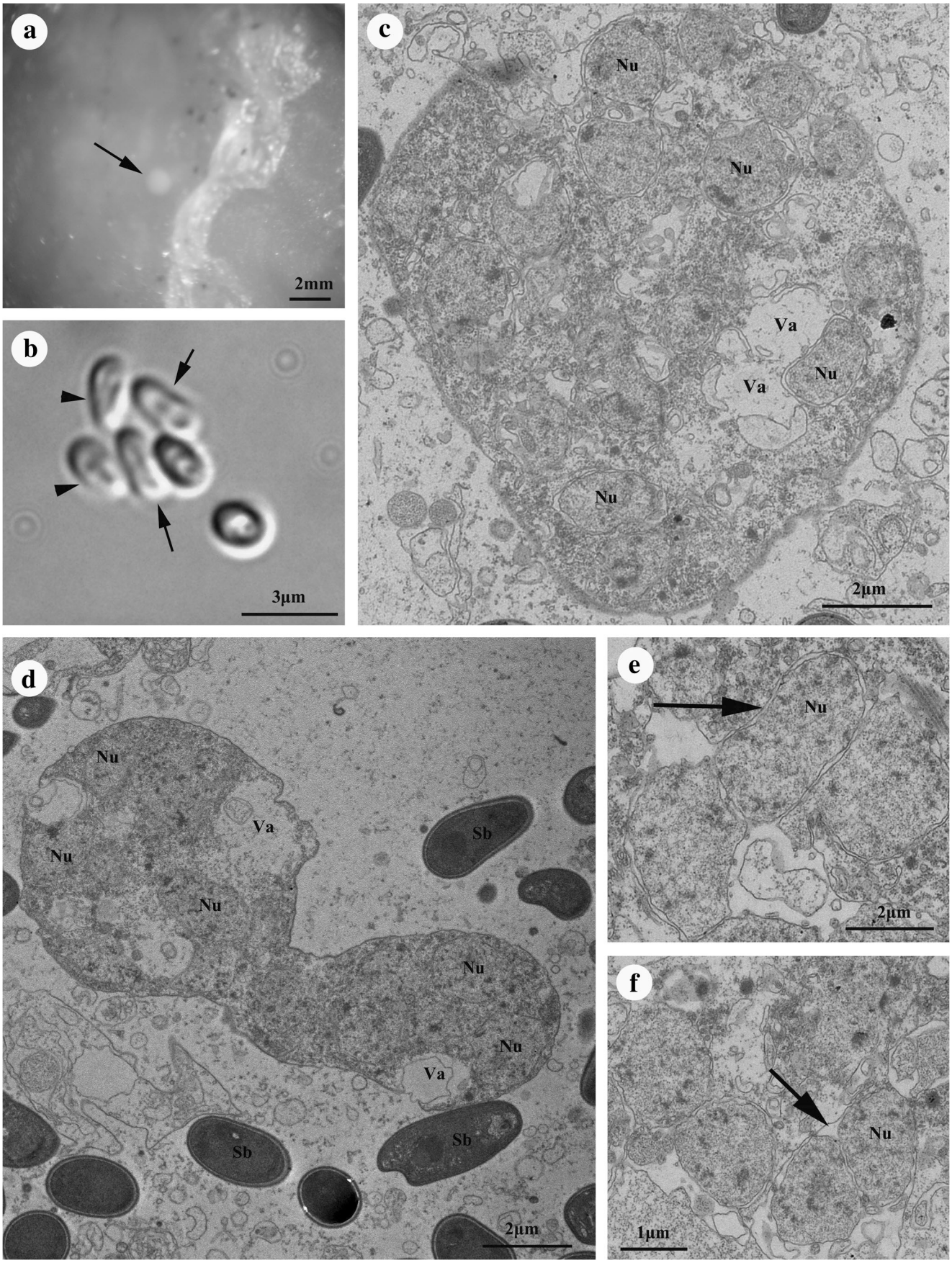
Transmission electron microscopy

For transmission electron microscopy (TEM), glutaraldehyde-fixed xenomas were washed in sodium cacodylate buffer twice (10 min) and placed into 1% osmium tetroxide (OsO4) solution for 1 h. After dehydration through a gradual ascending series of ethanol and propylene oxide series, samples were embedded in Spur resin. Ultrathin sections (70–90 nm) of these areas were mounted on uncoated copper grids and stained with uranyl acetate and lead citrate. Sections were examined using a Hitachi HT-7700 TEM.

DNA extraction, PCR, and sequencing

Four ethanol-fixed xenomas isolated from the infected liver of two fish were washed with distilled water 2 times to remove all ethanol. Genomic DNA was extracted by a commercial tissue extraction kit (Qiagen, Hilden, Germany). For amplification of SSU rDNA, V1f (5′-CACCAGGTTGATTCTGCC-3′) and 1492r (5′-GGTTACCTTGTTACGACTT-3′) primers were used (Nilsen 2000; Vossbrinck and Debrunner-Vossbrinck 2005). The PCR was carried out in a 50 μl reaction mixture containing PCR buffer, 200 mM dNTP, 2 mM $MgCl_2$, 1.25 units Taq polymerase, 20 pm each primer, and 2 μl DNA template. Thermocycler parameters were as follows: an initial denaturation step at 95 °C for 4 min, followed by 35 cycles of denaturation at 94 °C for 1 min, annealing at 46 °C for 30 s, elongation at 72 °C for 2 min, and a final extension at 72 °C for 10 min. The target PCR products were

Fig. 1 Photomicrographs of *Pseudokabatana alburnus* n. gen. n. sp. by light and transmission electron microscopy. **a** A whitish xenoma (arrow) at the surface of liver of host. **b** Fresh spores released from a xenoma, showing some normal spores (arrowhead) and slightly aberrant spores (arrow). **c** A rounded merogonal plasmodium contained numerous nuclei (Nu) and some vacuoles (Va). **d** A merogonal plasmodium surrounded by numerous sporoblasts (Sb). **e** A dividing nucleus (arrow). **f** Late division stage of an isolated nucleus to two unpaired nuclei (arrow)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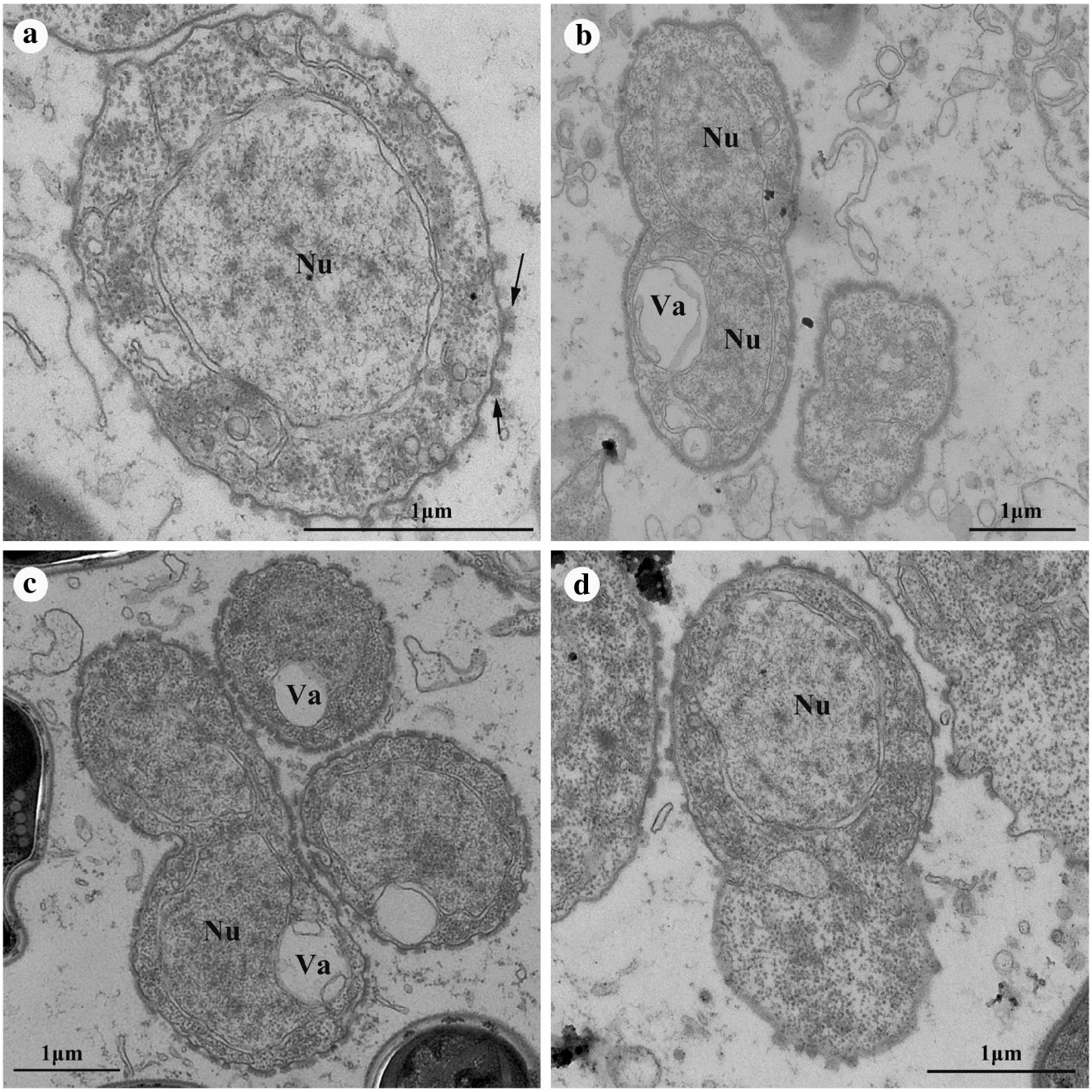

Fig. 2 Electron micrographs of sporogony of *Pseudokabatana alburnus* n. gen. n. sp. by transmission electron microscopy. **a** A sporont with isolated hypertrophied nucleus (Nu) located centrally and numerous electron-dense incrustations on the cell coat. **b**, **c** Sporonts in the division process to evolve into disporoblasts, some of them with a vacuole (Va). **d** An elongating early sporoblast

excised from an agarose gel and purified using a PCR purification kit (CWBiotech, Beijing, China) and cloned into PMD-18T vector system (Takara, Tokyo, Japan). Three positive clones (two were from one fish and the third from the other) were randomly selected and each sequenced using the vector primers (M13F(-47) and M13R(-48)) in both directions with the ABI BigDye Terminator v3.1 Cycle Sequencing Kit with an ABI 3100 Genetic Analyzer.

Molecular analysis

Sequences of each clone were assembled by BioEdit (Hall 1999). A BLASTN search was used to determine whether the obtained consensus sequence was a microsporidium and to compare to the sequences with highest similarity. Sequences with high similarity and others of interest from across Clade 5 of the Microsporidia were retrieved from the GenBank database

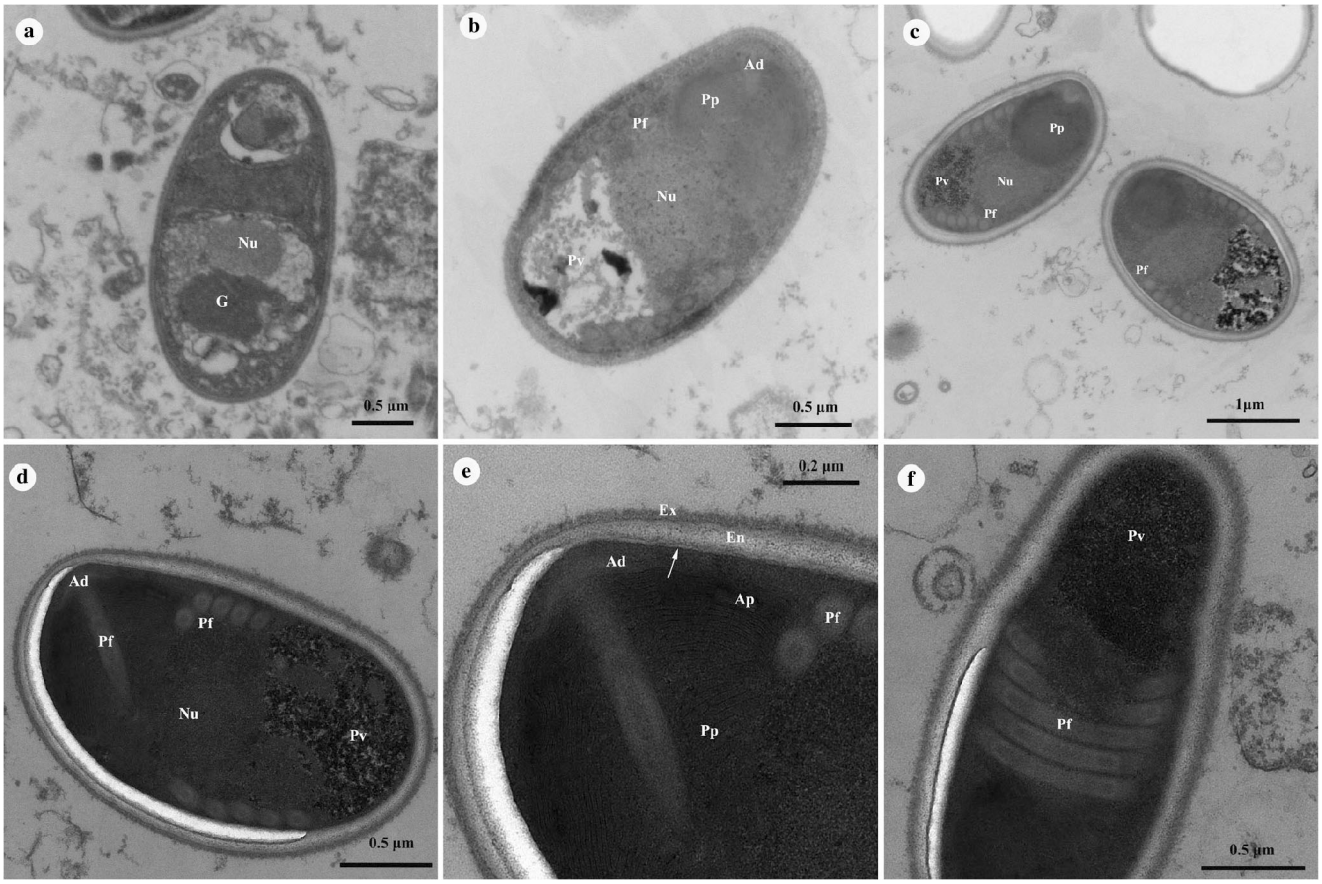

Fig. 3 Electron micrographs of spores of *Pseudokabatana alburnus* n. gen. n. sp. **a** An immature spore, showing a dense globular inclusion (G) in posterior vacuole. **b** An immature spore, showing the presence of extrusion apparatus. **c** Two mature spores with 5 (left) and 6 (right) coils of polar filaments, respectively. **d** Longitudinal section of a spore, showing three layered spore wall and extruding apparatus. **e** Detail of the anterior region of a spore, showing the anchoring disc (Ad) of eccentrically locating, bipartite lamellar polaroplast and trilaminar spore wall composed of thin exospore (Ex), thick endospore (En) and plasmalemma (arrow). **f** Transverse section of a mature spore showing the detail of polar filaments. (Ad) anchoring disc. (Nu) nucleus. (Ap) anterior polaroplast. (Pp) posterior polaroplast. (Pf) polar filaments. (Pv) posterior vacuole

(Stentiford et al. 2018). In total, 40 sequences were aligned using the Clustal X default setting (Thompson et al. 1997). The alignment was corrected manually using the alignment editor function within MEGA 6.0 (Tamura et al. 2013). Distance estimation was performed using the Kimura-2 parameter model distance matrix for transitions and transversions. Phylogenetic analyses were conducted using the maximum likelihood (ML) method in PhyML 3.0 and Bayesian inference (BI) in Mr. Bayes, respectively (Guindon et al. 2010; Ronquist and Huelsenbeck 2003). The optimal evolutionary model for ML and BI was GTR + I + G as determined by jModelTest 3.07 (Posada 2008) using the Akaike information criteria. *Amblyospora stimuli* (AF027685) was used as an outgroup to root the tree. Two independent runs were conducted with four chains for one million generations for BI. Phylogenetic trees were sampled every 100 generations. The first 25% of the samples were discarded from the cold chain (burninfrac = 0.25). Bootstrap confidence values were calculated with 100 replicates for ML. Support values of below 50 were not shown. Trees were initially examined in TreeView (Page 1996), and then edited and annotated in Adobe Illustrator (Adobe System, San Jose, CA, USA).

Results

Macroscopical and light microscopical observations

Xenomas were detected in the liver of 4 of 8 *C. alburnus* examined (apparent infection prevalence 50%; Fig. 1a). The xenomas were spherical, whitish-opaque in coloration, and up to 1.2 mm in diameter. After rupturing xenomas, large numbers of typical microsporidian spores, with a low proportion of apparently aberrant spores were liberated (Fig. 1b). The majority of spores were of a single type, pyriform in shape, and were not bound within an obvious sporophorous vesicle.

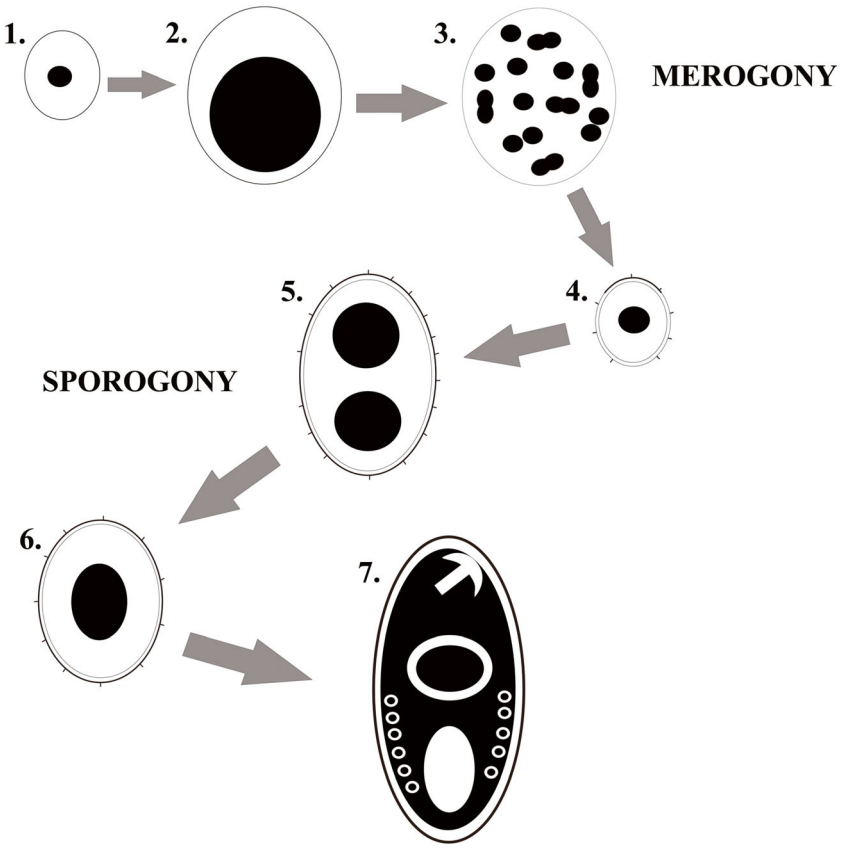

Fig. 4 Putative life cycle of *Pseudokabatana alburnus* n. gen. n. sp. inferred from the ultrastructural characteristics of developmental stages. (1) Uninucleate meront. (2) Enlarged uninucleate meront. (3) Multinucleate merogonal plasmodium. (4) Early stage of uninucleate sporont. (5) Disporoblasts. (6) Uninucleate sporoblast. (7) Mature Spore

Ultrastructural observation

Transmission electron microscopy revealed numerous life stages of a microsporidian parasite contained within dissected xenomas. Development within the xenoma was apparently asynchronous with simultaneous presence of merogonal plasmodia, sporonts, sporoblasts, and mature spores (Fig. 1c, d). In all cases, parasite cells occurred in directed contact with the host cell cytoplasm and contained isolated nuclei (unikaryotic). The earliest observed stages were multinucleated merogonal plasmodia, containing up to 20 monokaryotic nuclei, and surrounded by an amorphous electron-dense membrane (Fig. 1c). Merogonal plasmodia underwent plasmotomy in apparent synchrony with division of their nuclei (Fig. 1d–f), leading to formation of uninucleate sporonts which progressively acquired an electron-dense endospore decorated with further electron-dense bodies, presumably formed from the amorphous material that had surrounded the merogonal plasmodium. These bodies remained visible through further maturation of the sporont to a sporoblast until eventual formation of the mature spore (Fig. 2a–d). In some cases, uninucleate sporonts appeared to undergo further binary fission to form bi-nucleate sporonts (and then presumably uninucleate sporoblasts), rather than transforming directly into sporoblasts. Electron-lucent vacuoles could be generally observed in merogonal plasmodia (up to 2.22 μm in diameter) and disporoblasts (up to 1.12 μm in diameter) (Figs. 1c, d and 2b, c). Maturation of uninucleate sporoblasts to mature spores involved the elongation of sporoblasts and further differentiation of the spore extrusion organelles, including the anchoring disc, polaroplast, polar filament, and posterior vacuole (Fig. 3a–f). A dense globular inclusion was often observed in the posterior vacuole of immature spores (Fig. 3a), but disappeared in the mature spore. Mature spores were monokaryotic, pyriform, and measured 2.3 ± 0.19 μm long and 1.3 ± 0.10 μm wide ($N = 30$). Regular spores had an electron-dense exospore which was approximately 36–55 nm thick, an electron-lucent endospore that was 84–110 nm thick, and an internal plasma membrane. The endospore layer appeared slightly thinner in the vicinity of the anchoring disc, which located eccentrically in the anterior portion of the spores (Fig. 3d, e). Polar filaments were isofilar, coiled 5–6 turns, and arranged in one row, rarely two (Fig. 3b–f). The polaroplast was bipartite with tightly packed anterior lamellate membranes and a loosely aligned posterior lamellate region (Fig. 3d, e). The posterior vacuole occupied one third of spore length and was surrounded by polar filament coils (Fig. 3b, c). The proposed life cycle for the microsporidian infecting the liver of *C. alburnus* is shown in Fig. 4.

Table 1 Comparison of SSU rDNA sequences of *Pseudokabatana alburnus* n. gen. n. sp. and microsporidian species of high sequence identity: percentage of similarity (top diagonal) and pairwise distance (bottom diagonal) obtained by Kimura-2 parameter analysis

Species (GenBank accession number)	1	2	3	4	5	6	7	8	9	10	11	12	13
1 *Pseudokabatana alburnus* n. gen. n. sp. (MF974572)	–	*89.5*	*89.2*	*87.8*	*87.6*	*88.2*	*87.2*	*86.8*	*87.2*	*86.4*	*87.4*	*86.7*	*84.5*
2 *Kabatana rondoni* (FJ843105)	*0.105*	–	87.8	86.8	86.3	86.8	85.5	85.6	85.4	85.3	86.1	84.1	81.8
3 *Kabatana* sp. (EU682928)	*0.108*	0.122	–	93.8	92.1	98.4	90.5	90.2	90.5	90.0	91.8	90.0	88.0
4 *Kabatana takedai* (AF356222)	*0.122*	0.132	0.062	–	89.5	93.3	88.6	88.2	88.6	87.8	89.2	87.7	85.8
5 *Spraguea lophii* (AF033197)	*0.124*	0.137	0.079	0.105	–	91.2	95.9	94.5	95.8	94.0	99.7	94.0	92.0
6 *Kabatana* sp. (JQ062989)	*0.118*	0.132	0.016	0.067	0.088	–	90.2	89.3	90.2	89.1	90.9	89.4	87.3
7 *Spraguea* sp. (AB623034)	*0.128*	0.145	0.095	0.114	0.041	0.098	–	95.1	99.8	95.0	95.7	94.8	92.7
8 *Microgemma minius* (KJ865404)	*0.132*	0.144	0.098	0.118	0.055	0.107	0.049	–	95.1	99.0	94.2	94.1	92.0
9 *Spraguea* sp. (JQ820238)	*0.128*	0.146	0.095	0.114	0.042	0.098	0.002	0.049	–	94.9	95.6	94.8	92.7
10 *Microgemma* sp. (AJ252952)	*0.136*	0.147	0.100	0.122	0.060	0.109	0.050	0.010	0.051	–	93.8	93.8	91.8
11 *Spraguea gastrophysus* (GQ868443)	*0.126*	0.139	0.082	0.108	0.003	0.091	0.043	0.058	0.044	0.062	–	93.9	91.7
12 *Tetramicra brevifilum* (AF364303)	*0.133*	0.159	0.100	0.123	0.060	0.106	0.052	0.059	0.052	0.062	0.061	–	96.4
13 *Microgemma caulleryi* (AY033054)	*0.155*	0.182	0.120	0.142	0.080	0.126	0.073	0.080	0.073	0.082	0.083	0.036	–

Italicized entries were the similarities and distances of present species compared with other microsporidian species

Molecular analysis

The sequence obtained from amplification of the partial SSU rDNA gene was 1383 bp in length and identical among the three clones. Therefore, only one consensus sequence was deposited in GenBank under accession number MF974572. A homology search using the obtained SSU rDNA amplification products using BLAST revealed no identical match to any known microsporidian taxon sequence available in GenBank. Highest similarity occurred with *Kabatana rondoni*, a microsporidian infecting the skeletal muscle of *Gymnorhamphichthys rondoni* (Table 1). The pairwise distances/similarities calculated by Kimura 2-parameter model between the parasite from *C. alburnus* (this study) and those with highest sequence similarity within GenBank are given in Table 1 (distances/similarities ranging from 0.105/89.5% to 0.155/84.5%). Bayesian and maximum likelihood analyses of the aligned SSU rRNA genes generated highly similar topologies, although with different support values at some branch nodes, which positioned the present species within a cluster containing representative species from the genera *Kabatana*, *Microgemma*, *Spraguea*, *Tetramicra, Inodosporus*, and *Potaspora*. Within this cluster, the parasite from *C. alburnus* branched with *K. rondoni* within a *Kabatana* lineage, with a high support value (Fig. 5). However, the partial SSU rRNA sequence of the novel taxon was over 10% different across the sequenced region to *K. rondoni*. Based upon these differences, and those observed in type host, tissue tropism, formation of xenomas and, aspects of morphology, we propose erection of a new genus (*Pseudokabatana* n. gen) to contain the parasite from *C. alburnus* and further, erect a type species within this new genus, *Pseudokabatana alburnus* n. sp.

Discussion

The genetic diversity of the Microsporidia is likely considerably under described (Stentiford et al. 2013a; Williams et al. 2018). Despite their propensity to infect a vast range of eukaryote taxa (including single-celled organisms), relatively little focus has been given to hosts residing in global fresh waters. In China, despite sporadic reports over the past century of microsporidian parasites in freshwater fish (Chen 1955, 1956a, b; Chen and Xie 1960; He and Li 1985; He 1988), the true diversity is significantly under-reported.

In this study, we utilized morphology, ultrastructure, and molecular phylogenetic data to describe the first microsporidian parasite in fish from the sub-family Cultrinae (Family Cyprinidae). *Pseudokabatana alburnus* n. gen. n. sp. infects the liver of the topmouth culter (*Culter alburnus*) where it leads to xenoma formation visible at autopsy, in half of the fish sampled from Lake Poyang, China.

Morphological characters of *P. alburnus* resemble those observed in members of the genus *Kabatana*, namely, possession of isolated (unikaryotic) nuclei throughout the whole life cycle, development stages in direct contact with the host cytoplasm, and a similar merogonic and sporogonic development pattern (Lom et al. 1999). However, differences between *P. alburnus* and the nine described *Kabatana* spp. (*K. arthuri*, the type species, *K. takedai*, *K. seriloae*, *K. newberryi*, *K. rondoni* and four unidentified *Kabatana* species; Lom and Nilsen 2003; Kent et al. 2014) also exist (see Table 2). The novel taxon differs from *K. newberryi* and *K. rondoni* by its smaller size, as well as fewer turns of the polar filament within mature spores (Casal et al. 2010; McGourty et al. 2007). *Kabatana arthuri*

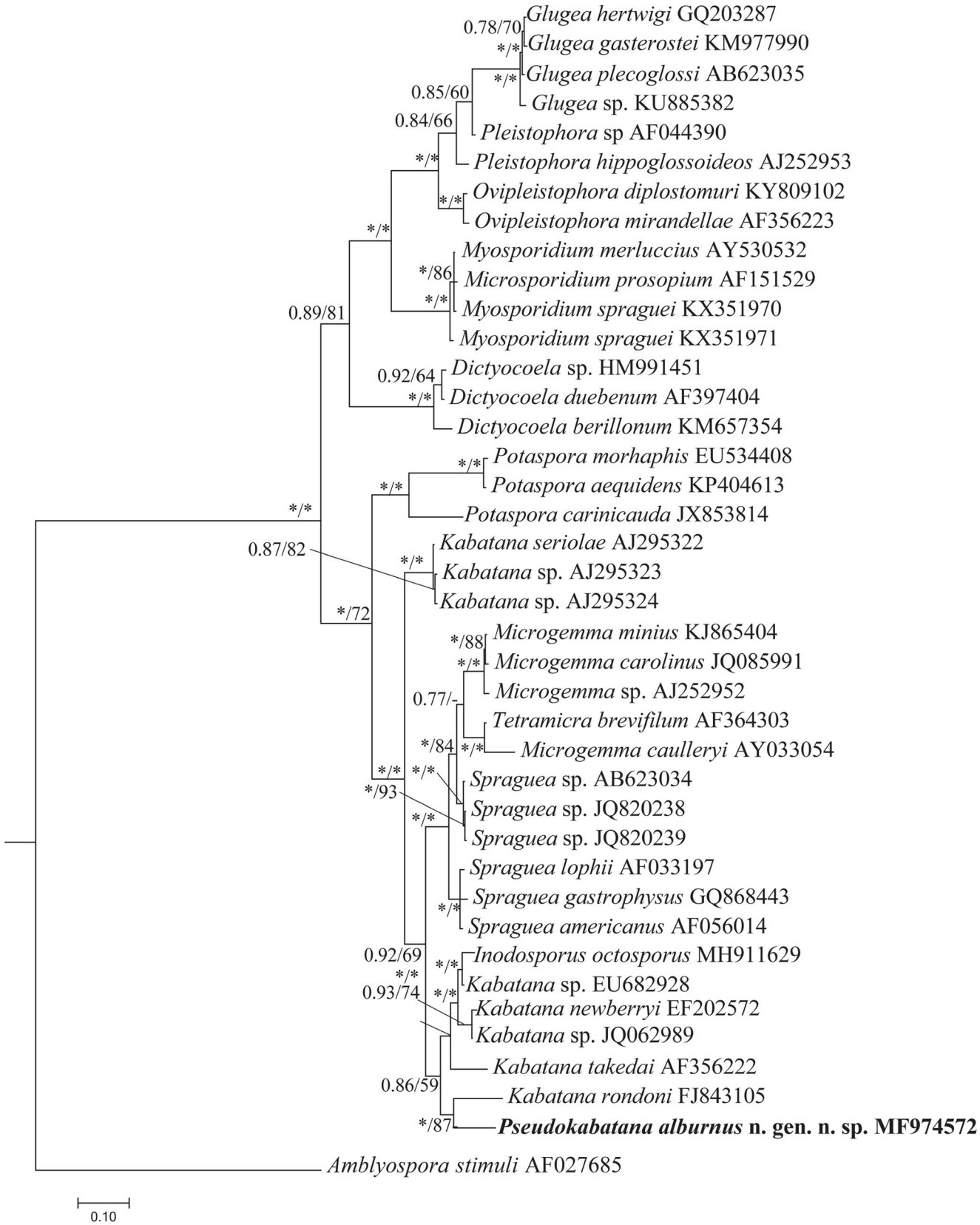

Fig. 5 Small subunit ribosomal RNA gene-based phylogeny of *Pseudokabatana alburnus* n. gen. n. sp. and the aligned microsporidian species estimated by Bayesian Inference (BI) method. *Amblyospora stimuli* was used as an outgroup. The species names are followed by GenBank accession number. BI posterior probabilities are shown first, followed by ML support values on the branch nodes. Asterisks indicate support values > 0.95 or 95% and dashes indicate values < 0.50 or 50%, respectively. The present species was indicated in bold

Table 2 Comparison of *Pseudokabatana alburnus* n. gen. n. sp. with the most closely morphological related *Kabatana* spp.

Species	Host	Habitat	Infection sites	SS	SL	SW	PF(rows)	References
Pseudokabatana alburnus n. gen. n. sp.	*Culter alburnus*	Freshwater	Liver	Pyriform	2.3 ± 0.19[a]	1.3 ± 0.1	5-6 (1)	Present study
K. rondoni	*Gymnorhamphichthys rondon*	Freshwater	Skeletal muscles	Pyriform	4.25 ± 0.38	2.37 ± 0.42	8–10 (2)	Casal et al. (2010)
K. newberryi	*Gobiusculus flavescens*	Brackish	Skeletal muscles	Ovoid	2.8[b] (2.5–3.1)[c]	1.9 (1.5–2.3)	9–10 (1–2)	McGourty et al. (2007)
K. takedai	*Oncorhynchus masou*	Freshwater	Skeletal muscles	Ovoid	3.3 (4.5–6.2)	1.9 (1.6–2.1)	3–4 (1)	Lom et al. (2001)
K. seriloae	*Seriola quinqueradiata*	Marine	Skeletal muscles	Ovoid	3.3	2.2	4–5 (1)	Lom et al. (1999)
K. arthuri	*Pangasius sutchi*	Freshwater	Skeletal muscles	Pyriform	3.1 (2.2–4.2)	1.9 (1.5–2.4)	5–6 (1)	Lom et al. (1999)

SS spore shape, *SL* spore length, *SW* spore width, *PF* polar filaments

[a] Mean ± SD

[b] Mean

[c] Minimum-maximum

and *K. takedai* also possess 3–6 turns of the polar filament; however, their mature spores are larger than that observed in our study of *P. alburnus*. In addition, their multinucleate meronts are cylindrical and not spherical, as observed for *P. alburnus* (Lom et al. 1990, 1999, 2001). *Kabatana seriloae* infects the marine fish host *Seriola quinqueradiata* (Bell et al. 2001) whereas *P. alburnus* infects a freshwater host. All previously reported *Kabatana* species infect the skeletal musculature of their fish hosts and have not been known to elict the formation of xenomas. In contrast, *P. alburnus* infects the liver and forms distinctive xenomas visible on the surface of the organ at autopsy. In addition to these differences in environmental niche, tissue tropism, and morphology, the similarity in partial SSU rDNA sequence between *P. alburnus* and its closest branch relative (*Kabatana rondoni*) is only 89.5%, lower than intragenus similarity of most genera of Microsporidia (Vossbrinck and Debrunner-Vossbrinck 2005). Taken together, a new genus, *Pseudokabatana*, was proposed to contain the type species *P. alburnus*. Our analysis also supports previously published works which shows that *Kabatana* is not monophyletic, with proposed members of the genus interspersed with parasites from genus *Microgemma*, *Tetramicra*, *Spraguea*, and *Indosporus* (McGourty et al. 2007; Casal et al. 2010, 2012; Stentiford et al. 2018). Only one genus from this clade (*Microgemma*) has been reported to infect the liver of its marine fish host. However, compared to *P. alburnus*, *Microgemma* possesses a different sporogenic sequence to that observed in our study (Ralphs and Matthews 1986) (Fig. 4). Furthermore, the closest *Microgemma* branch relative to *P. alburnus*, *M. minus* showed only 86.8% SSU rDNA sequence similarity to the novel taxon. Previous work, focused on potential for significant plasticity in morphology of even closely related microsporidians, has demonstrated the importance of utilizing robust genomic characters when describing new taxa (Stentiford et al. 2013b).

In conclusion, the comprehensive comparative analysis of ecological, morphological, ultrastructural charateristics, and phylogenetic characteristics supports the erection of a novel genus within clade 5 microsporidians (including *Kabatana*, *Inodosporus*) between fish and invertebrate hosts (Stentiford et al. 2018). Further studies are now required to investigate the presence of microsporidia in benthic invertebrates inhabiting Lake Poyang.

Taxonomic summary

Genus *Pseudokabatana* n. gen.

Definition: Xenoma formation in the liver of host. Isolated nuclei throughout all developmental stages. Direct contact of parasite life stages with host cell cytoplasm. Segmentation of multinucleate merogonal plasmodia via plasmotomy. Uninucleate cells emanating from multinucleate plasmodia have potential for further nuclear division and division to form uninucleate sporoblasts. Electron-dense incrustations define development of the sporont. Sporoblasts develop directly to form mature uninucleate spores.

Type species: *Pseudokabatana alburnus* n. gen. n. sp.

Description: Xenoma formation. Spores, monotypic and pyriform, approximately 2.3 ± 0.19 × 1.3 ± 0.10 μm in size. Polar filaments coiled with 5–6 turns arranged in one row. All life stage monokaryotic and developing in direct contact with host cell cytoplasm. Sporogony involving a disporoblasts possible before formation of unikaryotic sporoblast and mature spore. Spore wall is trilaminar with thin electron-dense exospore, a thick electron-lucent endospore, and a plasma membrane.

Diagnosis: Presence of a microsporidium with descriptive typical characteristics of the genus in the cytoplasm of liver of host. Diagnosis of morphological features by light and transmission electron microscopy. Nucleic acid-based diagnosis with PCR amplification, analysis of SSU rRNA gene sequence, and comparison to GenBank accession number MF974572.

Type host: topmouth culter *Culter alburnus* Basilewsky, 1855

Type locality: Lake Poyang off Xingzi county, Jiangxi province, China

Site of infection: Hepatocytes of the liver

Etymology: The generic name relates to proximity of the novel taxon to the closest branch relative (*Kabatana*). The specific epithet relates to host species name.

Type material: Syntype specimens of TEM resin blocks deposited in the Museum of Hydrobiological Sciences, Institute of Hydrobiology, Chinese Academy of Sciences MTR20160715, China. The partial SSU rDNA sequence was deposited in the GenBank under accession number of MF974572.

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