Journal of Invertebrate Pathology 105 (2010) 366–370

Contents lists available at ScienceDirect

Journal of Invertebrate Pathology

journal homepage: www.elsevier.com/locate/jip

Short Communication

Phylogenetic position of *Octosporea muscaedomesticae* (Microsporidia) and its relationship to *Octosporea bayeri* based on small subunit rDNA analysis

Charles R. Vossbrinck [a,*], Michael D. Baker [b], Theodore G. Andreadis [c]

[a] Department of Environmental Science, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, USA
[b] DNA Sequencing and Synthesis Facility, Office of Biotechnology, Iowa State University, Ames, IA 50011, USA
[c] Center for Vector Biology & Zoonotic Diseases, The Connecticut Agricultural Experiment Station, 123 Huntington Street, New Haven, CT 06511, USA

ARTICLE INFO

Article history:
Received 5 April 2010
Accepted 26 July 2010
Available online 3 August 2010

Keywords:
Octosporea muscaedomesticae
Microsporidia
Phormia regina
Diptera
Phylogeny
Neoflabelliforma

ABSTRACT

Comparative phylogenetic analysis of the small subunit rDNA sequence of *Octosporea muscaedomesticae* (Flu, 1911) (type species) (Microsporidia) isolated from the blowfly *Phormia regina* (Diptera:Calliphoridae) is presented. Neighbor Joining bootstrap, Maximum Parsimony and Maximum Likelihood analyses with 38 microsporidian taxa representing five major clades of Microsporidia placed *O. muscaedomesticae* on a separate branch within a clade containing parasites of freshwater hosts. *O. muscaedomesticae* differed from *Octosporea bayeri*, a parasite of the microcrustacean, *Daphnia magna* (Cladocera:Daphniidae) by 29% demonstrating that the latter microsporidium is not closely related to the type species at the generic level, and should not be placed within the genus *Octosporea*, a conclusion that is further supported by morphological and developmental differences. Considering the number of disparately related hosts from which *Octosporea* species have been previously described based mostly on developmental and morphological characters it is likely that many will not fit the current definition of the genus, and it is possible that molecular analysis of these species will show that this genus as defined represents a polyphyletic grouping of unrelated taxa.

1. Introduction

The genus *Octosporea* was created in 1911 by Flu to describe a microsporidium, *Octosporea muscaedomesticae* (Flu, 1911) from midgut epithelium of adult stages of the common housefly *Musca domestica* (Diptera:Muscidae) (Flu, 1911). The genus presently contains 12 recognized species (Sprague, 1977) and is defined as having presporulation and sporont stages with nuclei in the diplokaryotic arrangement, and octosporoblastic sporogony wherein diplokaryotic sporogonial plasmodia divide by multiple fission (rosette formation) to produce eight binucleate, ovocylindrical spores within a sporophorous vesicle (Sprague et al., 1992; Becnel and Andreadis, 1999).

The type species, *O. muscaedomesticae* has a comparatively broad host range among calypterate muscoid flies. In addition to *M. domestica*, naturally acquired infections have been found in field-collected *Calliphora erthrocephala*, *Calliphora vomitoria*, *Cochliomyia macellaria*, *Musca sorbans*, *Phaenicia sericata*, *Phormia regina*, and *Pollenia ruduis* (Thompson, 1935; Fantham and Porter, 1958; Kramer, 1964), and this microsporidium has been experimentally transmitted to *Fannia scalaris*, *Lucilia cuprina*, *Musca autumnalis*, *Muscina stabulans*, and *Sarcophaga bullata* (Kramer, 1973; Sprague, 1977; Cooper et al., 1983). *O. muscaedomesticae* has also been reported from or found infectious to three species of *Drosophila* (*Drosophila confusa*, *Drosophila melanogaster*, *Drosophila plurilineata*), the Mediterranean fruit fly, *Ceratitis capitata*, and the anthomiid fly, *Hylema antiqua* (Chatton and Krempf, 1911; Kramer, 1964; Ormieres et al., 1976). The isolate obtained from field-collected *P. regina* in Urbana, Illinois from 1960 to 1964 (Kramer, 1964, 1968a) and currently held within the frozen microsporidial depository at the Illinois Natural History Survey, has become the working isolate considered to be the defacto *O. muscaedomesticae* for a number of studies (Kramer, 1964, 1966, 1968a,b, 1972, 1973; Teetor-Barasch, 1979), and has been evaluated for the control of the Australian sheep blowfly, *Lucilia cuprinia* (Cooper et al., 1983; Smallridge et al., 1995).

The 11 other species of *Octosporea* have been described from a very diverse range of insect hosts including Collembola, other Diptera (Chaoboridae, Chironomidae, Drosophilidae, Faniidae, and Simulidae), Ephemeroptera, Hemiptera, and Lepidoptera, and from two freshwater microcrustaceans, *Gammarus duebeni* (Amphipoda) and *Daphnia magna* (Cladocera) (Sprague, 1976; Purrini and Weiser, 1983). The assignment of these 11 species to the genus *Octosporea* has been entirely based on morphological and developmental similarities with the type species as determined from light and less frequently, electron microscopy analyses.

* Corresponding author. Fax: +1 203 974 8502.
E-mail address: charles.vossbrinck@ct.gov (C.R. Vossbrinck).

0022-2011/$ - see front matter © 2010 Elsevier Inc. All rights reserved.
doi:10.1016/j.jip.2010.07.007

More recently, subunit ribosomal (ssrDNA) sequence data have been completed on *Octosporea bayeri*, a parasite of *D. magna* (GQ843833) (Corradi et al., 2009). A comparison of its ssrDNA, ITS, and 5′ end of the LSU have shown it to be nearly identical with *Flabelliforma magnivora* (AY649786) from the same host (Refardt and Ebert, 2006). The placement of *F. magnivora* within the genus *Flabelliforma* has been questioned. Based on molecular data, Canning et al. (2001) showed that *F. magnivora* was not closely related to the type species, *Flabelliforma montana* a parasite of the sandfly *Phlebotomus ariasi*, and suggested that the former species should not be placed in the genus. Morris and Freeman (2010) most recently reported ultrastructural and phylogenetic similarities between *F. magnivora* and a microsporidium, *Neoflabelliforma aurantiae* n. gen., n. sp. (GQ2016147) from the oligochaete, *Tubifex tubifex*, necessitating them to reassign *F. magnivora* as *Neoflabelliforma magnivora* n. comb.

In this investigation we now provide ssrDNA sequence data for the type species, *O. muscaedomesticae* and examine its phylogenetic relationship with *O. bayeri* and placement among other microsporidian taxa.

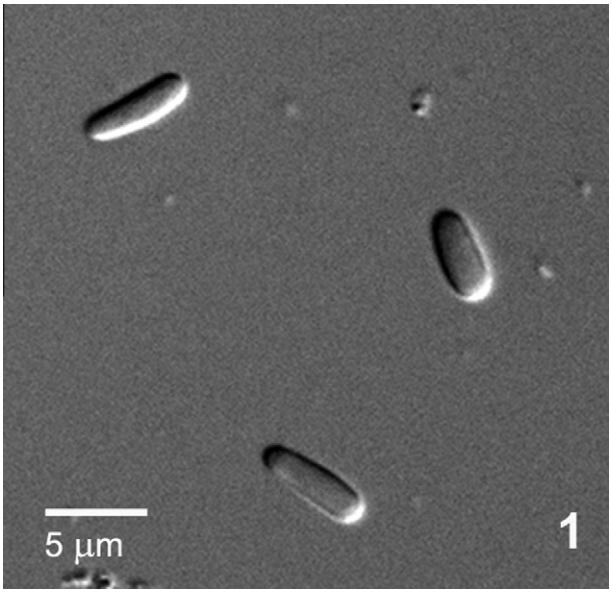

Fig. 1. Mature unfixed spores of *Octosporea muscaedomesticae* from *Phormia regina* as viewed under differential interference contrast (100× original magnification).

2. Materials and methods

Spores of *O. muscaedomesticae* were obtained from Leellen Solter at the Illinois Natural History Survey. The isolate was originally obtained from an infected laboratory colony of *P. regina* maintained within the Department of Entomology at the University of Illinois, Urbana–Champaign on August 2, 1988. Spores ($n = 20$) were measured and photographed with differential interference optics on a Zeiss Axioplan 2 Digital imaging system.

Spores were broken open by bead beating and DNA was amplified using primers as described previously (Baker et al., 1994, 1998). Sequencing was accomplished by the Sanger dideoxy method using radiolabeled P^{33} followed by polyacrylamide gel electrophoresis and autoradiography (Sambrook et al., 1982). Autoradiographs were read manually and entered into a MacIntosh Computer. Sequencing primers were those as previously reported (Baker et al., 1995, 1998; Vossbrinck and Andreadis, 2007). Sequences were aligned based on ssrRNA secondary structure by SequentiX corporation http://www.sequentix.de.

Bootstrap analysis was run using 1000 replicates using the Neighbor Joining/UPGMA search method. Maximum Likelihood and Maximum Parsimony analysis were accomplished with the Heuristic search method using the general search options with the PAUP software package (Swofford, 1999) running OS9 on a Macintosh Power PC.

3. Results and discussion

Spores were elongate, straight to slightly curved (Fig. 1). They had a mean length of 6.2 µm (SD = 0.65 µm) and a mean width of 2.5 µm (SD = 0.20 µm). These dimensions fall within the range of those reported previously (Kramer, 1964).

Fig. 2a and b represent Neighbor Joining bootstrap and Maximum Parsimony analyses of the ssrDNA sequence alignment for 38 microsporidian taxa representing five major clades of Microsporidia (Vossbrinck and Debrunner-Vossbrinck, 2005), *O. muscaedomesticae* (Accession Number FN794114) and *O. bayeri*, and two eukaryotic outgroups (*Basidiobolus ranarum* and *Tritrichomonas foetus*). In both analyses, as well as Maximum Likelihood analysis (not shown), *O. muscaedomesticae* was placed in the same relative position within Clade 1 (parasites of freshwater hosts), between *Marssoniella elegans* and *Trichotuzetia guttata* both parasites of cyclopoid copepods. This placement was well supported using Bootstrap analysis with an external probability support value of 100%.

As found previously (Refardt and Ebert, 2006), the sequences of *O. bayeri* and *N. magnivora* were nearly identical (1% difference). From this we infer that these two Microsporidia which infect the same crustacean host, *D. magna*, but from different geographic regions (Finland and England–Russia, respectively), are most likely conspecific with spore variation attributable to polymorphic development (Vizoso et al., 2005).

Most significantly, the sequence of *O. muscaedomesticae* differed from *O. bayeri* and *N. magnivora* by 29–30%, clearly demonstrating that the latter two Microsporidia are not closely related to the type species at the generic level, and based on our molecular analyses should not be placed within the genus *Octosporea*. This conclusion is further supported by morphological and developmental differences. Unlike *O. muscaedomesticae*, *O. bayeri*/*N. magnivora* have isolated nuclei throughout at least one portion of their life cycle, undergo sporogony yielding 4–16, but mostly 12 sporoblasts, and produce three morphologically distinct spores, oval, pyriform (uninucleate), and elongate to slightly curved (Larsson et al., 1998; Vizoso et al., 2005).

The systematic placement of *O. bayeri*/*N. magnivora* continues to be problematic and may require revision. Canning et al. (2001) first reported that this microsporidium should not be placed within the genus *Flabelliforma*, and our analysis concurs by showing a 28% nucleotide difference (based on our alignment) from the type species, *F. montana*. Morris and Freeman (2010) suggested placing *F. magnavora* in the newly erected genus *Neoflabelliforma* along with the type species *N. aurentiae* based on developmental and phylogenetic similarities, but did note differences in spore morphology (size, shape and length of the polar filament). Our bootstrap Neighbor Joining analysis (Fig. 1a) indicate that *N. aurentiae* and *O. bayeri*/*N. magnivora* are sister taxa but with a relatively low bootstrap support value of 73%. On the other hand, Maximum Parsimony (Fig. 1b) and Maximum Likelihood analyses place *N. aurantiae* separately as a sister taxon to *O. bayeri*/*N. magnivora*

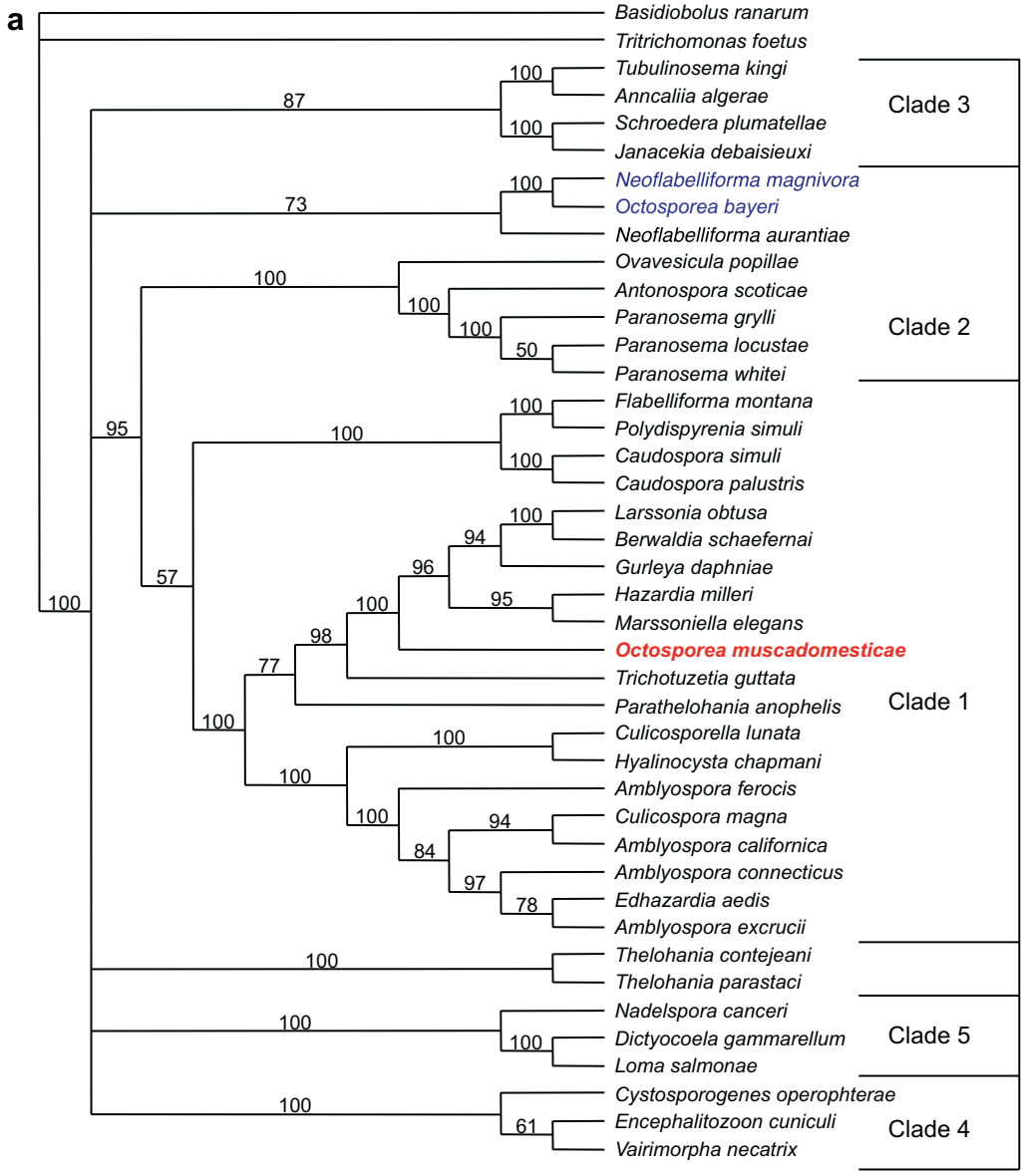

Fig. 2. Phylogenetic trees showing the relationship of *Octosporea muscaedomesticae* to 37 microsporidial taxa based on ssrDNA sequence data. (a) Neighbor Joining bootstrap analysis (1000 replicates); (b) one of two trees generated from a Maximum Parsimony analysis using the heuristic search method of PAUP.

and clades 4 and 5 with a high degree of sequence difference between these two species (28%).

Considering the number of disparately related hosts from which *Octosporea* species have been previously described based mostly on a limited number of developmental and morphological characters observed at the light microscope level it is likely that many will not fit the current definition of the genus (Sprague et al., 1992; Becnel and Andreadis, 1999). These include: *Octosporea carlochagasi*, host = *Pangstrongylus megistus* [Hemiptera]; *Octosporea chironomi* Weiser, 1943, host = *Camptochrionomus tentans* [Diptera:Chironomodae]; *Octosporea corethrae*, host = *Corethra* sp. [Diptera:Chaoboridae]; *Octosporea effeminans*, host = *Gammarus duebeni* [Amphipoda]; *Octosporea collembolae* (Purrini and Weiser, 1983), host = *Onychiurus quadricellatus* [Collembola]; *Octosporea ephestiae*, host = *Ephestia kuehniella* [Lepidoptera]; *Octosporea gammari*, host = *Gammarus pulex* [Amphipoda]; *Octosporea intestinalis*, host = *Rhithrogena semicolorata* [Ephemeroptera]; *Octosporea monospora*, host = *Drosphila confuse* [Diptera: Drosophilidae]; *Octosporea simulii*, host = *Simulium* sp. [Diptera:Simuliidae]; and *Octosprorea viridanae*, host = *Tortrix viridana* [Lepidoptera]) (Sprague, 1977; Purrini and Weiser, 1983). In addition, it is possible that molecular analysis of the aforementioned species will show that this genus as defined represents a polyphyletic grouping of unrelated taxa as we have demonstrated with *O. bayeri*. This has been shown with the *Nosema* clade (type species *Nosema bombycis*, host = [Lepidoptera]) which at one time included several species from unrelated hosts that have now been reassigned to new genera based on molecular analysis (e.g. *Nosema algerae* = *Anncaliia algerae*, host = *Anopheles stephensi* [Diptera]; *Nosema locustae*, host = *Locusta migratoria* [Orthoptera]; and *Nosema kingi* = *Tubulinosema kingi*, host = *Drosophila willistoni* [Diptera]).

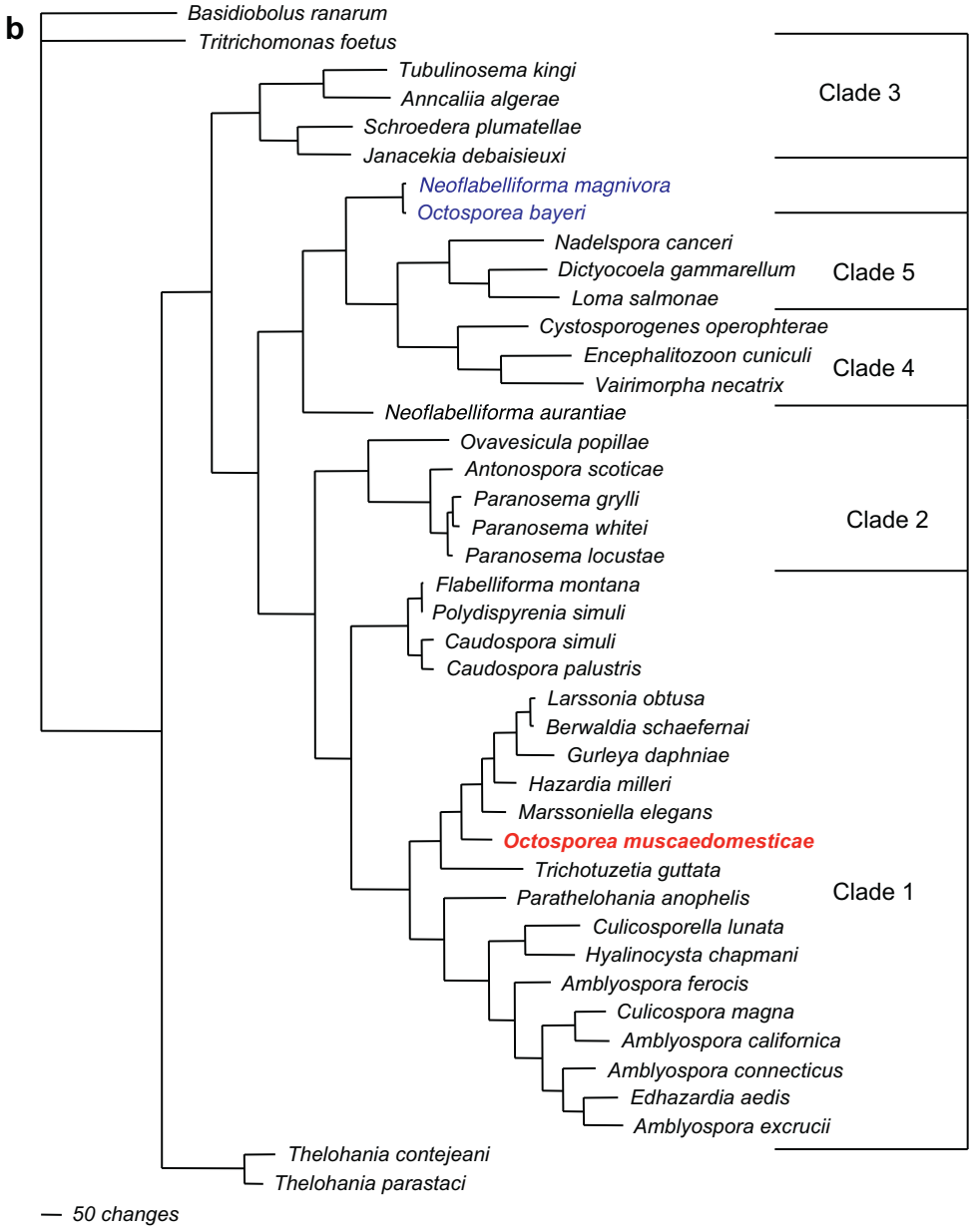

Fig. 2 (*continued*)

References

Baker, M.D., Vossbrinck, C.R., Maddox, J.V., Undeen, A.H., 1994. Phylogenetic relationships among *Vairimorpha* and *Nosema* species (Microspora) based on ribosomal RNA sequence data. J. Invertebr. Pathol. 64, 100–106.

Baker, M.D., Vossbrinck, C.R., Didier, E.S., Maddox, J.V., Shadduck, J.A., 1995. Small subunit ribosomal DNA phylogeny of various Microsporidia with emphasis on AIDS related forms. J. Eukaryot. Microbiol. 42, 564–570.

Baker, M.D., Vossbrinck, C.R., Becnel, J.J., Andreadis, T.G., 1998. Phylogeny of *Amblyospora* (Microsporida:Amblyosporidae) and related genera based on small subunit ribosomal DNA data: a possible example of host parasite cospeciation. J. Invertebr. Pathol. 71, 199–206.

Becnel, J.J., Andreadis, T.G., 1999. Microsporidia in insects. In: Witter, M., Weiss, L.M. (Eds.), The Microsporidia and Microsporidiosis. American Society of Microbiology Press, pp. 447–501.

Canning, E.U., Curry, A., Cheney, S.A., Lafranchi-Tristem, N.J., Ebert, D., Rifardt, D., Killick-Kendrick, M., Killick-Kendrick, R., 2001. *Flabelliforma montana* (Phylum Microsporidia) from *Phlebotomus ariasi* (Diptera, Psychodidae): ultrastructural observations and phylogenetic relationships. Eur. J. Protistol. 37, 207–221.

Chatton, E., Krempf, A., 1911. Sur le cycle evolutif et la position systematique des protistes du genre *Octosporea*, Flu, parasites des muscides. Bull. Soc. Zool. Fr. 36, 172–179.

Cooper, D.J., Pinnock, D.E., Bateman, S.M., 1983. Susceptibility of *Lucilia cuprina* (WIEDEMANN) (DIPTERA:CALLIPHORIDAE), to *Octosporea muscaedomesticae* FLU. J. Aust. Entomol. Soc. 22, 292.

Corradi, N., Haag, K.L., Pombert, J.-F., Ebert, D., Keeling, P.J., 2009. Draft genome sequence of the *Daphnia* pathogen *Octosporea bayeri*: insights into the gene content of a large microsporidian genome and a model for host–parasite interactions. Genome Biol. 10, R106.

Fantham, H.B., Porter, A., 1958. Some pathogenic bacteriform Microsporidia from Crustacea and Insecta. Proc. Zool. Soc. London 130, 153–168.

Flu, P.C., 1911. Studien über die im Darm der Stubenfliege, *Musca domestica*, vorkommenden protozoären Gebilde. Zentralbl. Bakteriol. Parasitenk. Infektionskr. Hyg. Abt. I Orig. 57, 522–535.

Kramer, J.P., 1964. The microsporidian *Octosporea muscaedomesticae* Flu, a parasite of calypterate muscoid flies in Illinois. J. Insect Pathol. 6, 331–342.

Kramer, J.P., 1966. An octosporeosis of muscoid flies caused by *Octosporea muscaedomesticae* Flu (Microsporidia). Am. Midl. Nat., 214–220.

Kramer, J.P., 1968a. An octosporeosis of the Black Blowfly *Phormia regina*: incidence rates of host and parasite. Z. f. Parasitenk. 30, 33–39.

Kramer, J.P., 1968b. An octosporeosis of the Black Blowfly *Phormia regina*: effect of temperature on the longevity of diseased Adults. Texas Rep. Biol. Med. 26, 199–204.

Kramer, J.P., 1972. The microsporidian *Octosporea muscaedomesticae* as a pathogen of larval and pupal *Phormia regina* (Diptera:Calliphoridae). J. NY Entomol. Soc. 3, 125–128.

Kramer, J.P., 1973. Susceptibility of sixteen species of muscoid flies to the microsporidian parasite *Octosporea muscaedomesticae*. J. NY Entomol. Soc. 81, 50–53.

Larsson, J.I.R., Ebert, D., Mangin, K.L., Vavra, J., 1998. Ultrastructural study and description of *Flabelliforma magnivora* sp. n. (Microspora: Duboscqiidae), a microsporidian parasite of *Daphnia magna* (Crustacea:Cladocera:Daphniidae). Acta Protozool. 37, 41–52.

Morris, D.J., Freeman, M.A., 2010. Hyperparasitism has wide-ranging implications for studies on the invertebrate phase of myxosporean (Myxozoa) life cycles. Int. J. Parasitol. 40, 357–369.

Ormieres, R., Baudoin, J., Brugerolle, G., Pralavorio, R., 1976. Ultrastructure de quelques stades de la microsporidie *Octosporea muscaedomesticae* Flu, parasite de *Ceratitis capitata* (Wiedemann) (Diptere, Trypetidae). J. Protozool. 23, 320–328.

Purrini, K., Weiser, J., 1983. *Octosporea collembolae* n.sp. (Microsporida:Microspora): a new microsporidian parasite of the springtail *Onychiurus quadricellatus* (Onychiuridae:Collembolae). J. Invertebr. Pathol 42, 135–142.

Refardt, D., Ebert, D., 2006. Quantitative PCR to detect, discriminate and quantify intracellular parasites in their host: an example from three microsporidians in *Daphnia*. Parasitology 133, 11–18.

Sambrook, J., Fritsch, E.F., Maniatis, T., 1982. Molecular Cloning a Laboratory Manual. Cold Spring Harbor Laboratory.

Smallridge, C.J., Cooper, D.J., Pinnock, D.E., 1995. The effect of the microsporidium *Octosporea muscaedomesticae* on Adult *Lucilia cuprina* (Diptera:Calliphoridae). J. Invertebr. Pathol. 66, 196–197.

Sprague, V., 1977. Systematics of the microsporidia. In: Bulla, L.A., Jr., Cheng, T.C. (Eds.), Comparative Pathobiology. Plenum Press, New York, pp. 1–510.

Sprague, V., Becnel, J.J., Hazard, E.I., 1992. Taxonomy of phylum microspora. Crit. Rev. Microbiol. 18, 285–395.

Swofford, D.L., 1999. PAUP 4.0: Phylogenetic Analysis using Parsimony (and other methods). Sinauer Associates, Sunderland, Mass.

Teetor-Barsch, G.E., Kramer, J.P., 1979. The preservation of infective spores of *Octosporea muscadomesticae* in *Phormia regina*, of *Nosema algerae* in *Anopheles stephensi*, and of *Nosema whitei* in *Tribolium castaneum* by *lyophilization*. J. Invertebr. Pathol. 33, 300–306.

Thompson, J.G., 1935. A microsporidian (*Octosporea*) in the intestine of *Musca sorbans* Wied. Trans. Roy. Soc. Trop. Med. 29, 6.

Vizoso, D.B., Lass, S., Ebert, D., 2005. Different mechanisms of transmission of the microsporidium *Octosporea bayeri*: a cocktail of solutions for the problem of parasite permanence. Parasitology 130, 1–9.

Vossbrinck, C.R., Andreadis, T.G., 2007. The phylogenetic position of *Ovavesicula popilliae* (Microsporidia) and its relationship to *Antonospora* and *Paranosema* based on small subunit rDNA analysis. J. Invertebr. Pathol. 96, 270–273.

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