Olpidiopsis bostrychiae: A new species of the endoparasitic oomycete that infects Bostrychia and other red algae

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Running title: Taxonomy and phylogeny of Olpidiopsis bostrychiae sp. nov.

KEY WORDS: Bostrychia, cox2, host specificity, red algae, sporogenesis, SSU rRNA, stramenopiles, transmission electron microscopy
ABSTRACT

*Olpidiopsis bostrychiae* sp. nov., a marine oomycete endoparasite which infects *Bostrychia* spp. and other red algae, was described and its phylogenetic position based on molecular data and ultrastructural morphology discussed. *Olpidiopsis bostrychiae* formed spherical to ellipsoidal unicellular thalli within the host cytoplasm. One or more sporangia developed in each infected host cell within 2-3 days of infection, depending on host species. The host cell remained alive until the thallus had acquired a fully differentiated cell wall. Premature death of the host cell also resulted in the aborted parasite development as well. The mature sporangium usually produced a single discharge tube. Zoospores with laterally inserted flagella developed within the mature sporangium. Based on whole cell morphological features and host specificity this *Bostrychia* parasite could be distinguished from all previously described *Olpidiopsis* parasites of red seaweeds and therefore was described as a new species. The ultrastructural features of this parasite are documented and compared and contrasted with other *Olpidiopsis* and oomycete species. Molecular phylogenetic trees inferred from both SSU rRNA gene and COII amino acid sequences showed that *O. bostrychiae* branched in the phylogenetic trees before the main saprolegnian and peronosporalean lineages within the monophyletic oomycete clade. In the SSU rRNA gene tree *Olpidiopsis bostrychiae* formed a monophyletic clade with *O. porphyrae*, a marine oomycete endoparasite which infects *Bangia* and *Porphyra* spp. However the tree also showed that there was a significant genetic distance between the two marine red algal parasitic *Olpidiopsis* species, suggesting considerable phylogenetic divergence within this genus which is also supported by differences in their ultrastructural appearance.
INTRODUCTION

Oomycetes (Peronosporomycetes) in the stramenopiles are morphologically and ecologically divergent organisms that live in marine, freshwater and terrestrial environments as saprophytes or parasites (Alexopoulos et al. 1996). They parasitize a wide range of animals (vertebrates and invertebrates), plants, algae and other protists (Sparrow 1960). Several oomycete taxa are known to be parasites of marine and freshwater algae, and ultrastructurral studies have been carried out on some species, such as Ectrogella perforans H. E. Petersen infecting marine diatom Licmophora hyalina C. Agardh (Raghukumar 1980a, b), Petersenia palmariae van der Meer & Pueschel infecting a marine red alga Palmaria mollis (Setchell & Gardner) van der Meer & Bird (Pueschel & van der Meer 1985), and Lagenisma coscinodisci Drebes infecting a marine diatom Coscinodiscus granii Gough (Schnepf et al. 1978a). Recently, combined ultrastructural and molecular studies on two oomycete marine algal endoparasites, Olpidiopsis porphyrae Sekimoto, Yokoo, Y. Kawamura & D. Honda infecting Porphyra C. Agardh and Bangia Lyngbye, and Eurychasma dicksonii (E. P. Wright) Magnus infecting wide range of brown algae, were carried out and showed that both species branched before the two main terrestrial oomycete clades (the Saprolegniales and Peronosporales) (Sekimoto et al. 2008a, b). This indicates the potential importance of these marine algal endoparasites in elucidating the phylogenetic origin and evolutionary development of this lineage.

The genus Olpidiopsis M. Cornu is one of the largest holocarpic oomycete genera, assigned in the order Lagenidiales (Sparrow 1960). They are all endobiotic parasites, which parasitize freshwater eucarpic oomycetes, chytrids, freshwater diatoms as well as marine green, brown and red algae (Sparrow 1960; Dick 2001). Recently, an Olpidiopsis species which infects the marine red algal species Bostrychia moritziana (Sonder ex Kützing) J. Agardh was isolated and its detailed host range was described (West et al. 2006). In this paper, we describe this parasite as a new species in the genus Olpidiopsis based upon the thallus morphology and host specificity and discuss phylogenetic relationships with other oomycete species based on ultrastructural morphology and molecular phylogenetic analyses as described recently for overall oomycete phylogeny in other marine genera (Sekimoto et al. 2007, 2008a,b).
MATERIALS AND METHODS

Cultures
Isolation and culture methods used for *Bostrychia* spp. were given in West (2005). *B. moritziana* 4439 infected with the parasite was collected from prop roots of the mangrove *Rhizophora* sp., Chenal d’Ampanarata, Belo sur Mer, Madagascar (20° 44.821’ S, 43° 59.654’ E) on 27 May 2004 as described by West et al. (2006). However, a second, more susceptible, host isolate (*B. moritziana* 4314) was used both for providing parasite material for transmission electron microscopy (TEM) and for isolating DNA for molecular phylogenetic analyses. The dual culture of the host and parasite was maintained in plastic Petri dishes in Provasoli ES medium (Provasoli 1968) prepared from filtered natural seawater (collected off Akashi, Hyogo, Japan) at 20 ± 1°C. They were illuminated with daylight-type fluorescent lamps at an irradiance of 12 μmol m⁻² s⁻¹ for 10 h per day. All cultures were transferred to fresh medium at 10 day intervals. The cultures of *B. moritziana* 4314 infected with *Olpidiopsis* (CCAP 4055/1) and uninfected host 4314 (CCAP 1357/8) were deposited into Culture Collection of Algae and Protozoa (CCAP, Oban, Scotland).

Host species susceptibility to infection with *Olpidiopsis bostrychiae*
The other potential host algae tested in the present study and their sources are given in Table 1. Some red algal isolates were previously used by West et al. (2006) and several isolates were newly tested in the present study. For this experiment, all isolates were maintained in IMR medium (Klochkova et al. 2006) or Provasoli ES medium in 90 × 15-mm plastic Petri dishes at 15°C, 15 μmol photons m⁻² s⁻¹ cool-white fluorescent lighting and 12:12 h light-dark regime. To maintain a combination of host and parasite, infected *B. moritziana* 4314 was added to cultures containing other potential host algae in 90 × 15-mm Petri dishes, and samples were examined daily with Olympus BX50 light microscope (Olympus, Tokyo, Japan).

Morphological observations
For light microscopy, living cells were observed with an Olympus BX60 light microscope fitted with differential interference contrast (Nomarski) objectives and recorded on a digital camera AxioCam HRc (Carl Zeiss, Oberkochen, Germany).
coupled to the AxioCam software.

For time-lapse video-microscopic observations, infected algal thalli were placed on a glass slide and a coverslip was lowered and sealed with VALAP (1:1:1; Vaseline: Lanolin: Paraffin) melted on a hot plate at 70°C. The slide preparations were examined on an Olympus BX-51 microscope under the oil immersion × 20 objective lens and recorded on a Digital Imaging Time-Lapse Recorder (Seoulin Bioscience, Seoul, Korea).

For transmission electron microscopy, infected algal thalli were fixed with 2.5% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2) containing 0.25M sucrose for 2 hours at ambient temperature. Following three 15 minute rinses in 0.1M phosphate buffer, the samples were post-fixed in 1% OsO₄ in buffer for 1 h at ambient temperature. Samples were then rinsed in 0.1M phosphate buffer and distilled water for 15 min for two times each, placed in a 1% uranyl acetate aqueous solution at ambient temperature for 1 hour, pre-embedded in 1% ultralow-gelling agarose (SeaKem GTG agarose, FMC, USA), and dehydrated in an acetone series (10% steps, 15 min each) at 4°C. Dehydration was completed by 5 changes of 100% dried acetone (30 min each). Samples were infiltrated and embedded in Quetol 651 resin (TAAB, Aldermaston, Berkshire, England) or Quetol 812 resin (Nissin EM, Tokyo, Japan), and polymerized at 60°C for 3 days. Blocks were sectioned with a Reichert Ultracut E ultra-microtome and collected on Formvar-coated copper slot grids. Ultrathin sections were initially stained for 15 min in 2% uranyl acetate, and then stained for 5 min in Reynolds’ lead citrate (Reynolds 1963), or for 30 min in Ultrostain 2 (Leica Microsystems, Wetzlar, Germany). Observations were made with Philips CM100 (FEI Company, Hillsboro, OR, USA) or Jeol JEM2000FX (JEOL Ltd., Tokyo, Japan) transmission electron microscopes operated at 80 kV.

Molecular phylogeny

Genomic DNA was extracted from both infected and uninfected cultures of *B. moritziana* 4314 isolate with the parasite. A healthy *B. moritziana* 4314 isolate was also extracted as a control. Approximately 100 mg of algal tissue was desiccated with sterilized silica beads in a 15 ml plastic tube, frozen in liquid nitrogen, and total DNA was extracted using the GenomicPrep Cells and Tissue DNA Isolation Kit (GE Healthcare UK, Buckinghamshire, England) as described by Sekimoto et al. (2008a).
Purified DNA was suspended in 30 μl TE.

The eukaryote-universal SR1 (Nakayama et al. 1996), the eukaryote-universal 18S13 (Honda et al. 1999), oomycete-specific peronosporomycete forward (Hudspeth et al. 2000) and oomycete-specific peronosporomycete reverse (Hudspeth et al. 2000) primers were used to amplify the parasite SSU RNA and cox2 genes, respectively. The following polymerase chain reaction (PCR) programs were used. An initial denaturation at 94˚C for 2 min was followed by 35 cycles of denaturation at 95˚C for 30 sec, annealing at 55˚C for 30 sec (SSU rRNA) / 50˚C for 30 sec (cox2), extension at 72˚C for 3 min (SSU rRNA) / 72˚C for 1 min 30 sec (cox2) and then a final extension at 72˚C for 5 min. TaKaRa rTaq or TaKaRa EX Taq (Takara Biomedicals, Otsu, Japan) were used as a DNA polymerase for PCR amplifications of SSU RNA gene region and of cox2 gene region, respectively. The PCR products were checked on a 1.4% TBE or 0.6% TAE agarose gels. They were then TA cloned using the QIAGEN PCR Cloning Kit (Qiagen, Hilden, Germany) in Escherichia coli. Different clones (host Bostrychia and parasite Olpidiopsis) were distinguished by their restriction patterns obtained by a combined EcoRI and EcoRV digestion as follows. Each E. coli colony containing TA cloned PCR products of host or parasite SSU RNA or cox2 genes was directly amplified with primers T7 (TAATACGACTCACTATAGGG) and SP6 (ATTTAGGTGACACTATAGAA), both of which were designed for the vector DNA used in this study (pDrive Cloning Vector in QIAGEN PCR Cloning Kit). Each amplified PCR product was mixed with restriction enzymes and buffer solution [0.5 μl EcoRI (15 units/μl), 0.5 μl EcoRV (15 units/μl), 0.5 μl 10 × H buffer (100 mM Tris-HCl, pH 7.5, 100 mM MgCl2, 10 mM Dithiothreitol, and 1000 mM NaCl), 5 μl PCR product, and 3.5 μl sterilized distilled water] and incubated at 37˚C overnight. Digested PCR products were checked on a 1.4% TBE agarose gel. Plasmid DNA of positive (parasite) clones was purified using the QIAprep Spin Miniprep Kit (Qiagen) and then sequenced using a primer-walking approach with BigDye Terminator v3.1 Cycle Sequencing Kit on a DNA autosequencer 310 (Applied Biosystems, Foster City, CA, USA).

Our SSU rRNA gene alignment was based on the aligned data set described in Küpper et al. (2006). Our sequences and the following SSU rRNA gene sequences from GenBank were individually added to the aligned data set through a profile alignment process by ClustalW, version 1.81 (Thompson et al. 1994): Aphanomyces invadans
Willoughby, R.J. Roberts & Chinabut (AF396684), Eurychasma dicksonii Eury96
(AY032607: Amended by Sekimoto et al. 2008b), Eurychasma dicksonii Eury05
(AB368176), Haliphthoros milfordensis Vishniac NJM0131 (AB178868), Haptoglossa
sp. (EU271966), Hyaloperonospora parasitica (Persoon) Constantinescu (AY742752),
Olpidiopsis porphyrae (AB287418), Peronophthora litchii C.C. Chen ex W.H. Ko,
H.S. Chang, H.J. Su, C.C. Chen & L.S. Leu (AY742750), Phytophthora megasperma
Drechsler (M54938), Pirsonia punctigerae Schweikert & Schnepf (AJ561115), Pirsonia
verrucosa Kühn (AJ561113), Plasmopara viticola (M. J. Berkeley & M.A. Curtis) A. N.
Berlese & De Toni (AY742754), Pseudoperonospora cubensis (M. J. Berkeley & M.A.
Curtis) Rostovzev (AY742760), Pythium aphanidermatum (Edson) Fitzpatrick
(AY742755), Pythium insidiosum De Cock, L. Mendoza, A.A. Padhye, Ajello &
Kaufman (AF442497), Rhizidiomyces apophysatus Zopf (AF163295), Saprolegnia
parasitica Coker (AB086898), and abalone endoparasite NJM0034 (AB178865). The
alignment was manually refined.

The cox2 gene alignment was based upon the aligned data set in Sekimoto et al.
(2007). The sequences reported here and the following sequences from GenBank were
also individually added to the aligned data set through a profile alignment process:
Albugo achyranthis (P. C. Hennings) Miyabe (AY913807), Albugo bliti
(Bivona-Bernardi) Kuntze (AY913805), Albugo candida (Persoon) Roussel
(DQ418522), Eurychasma dicksonii Eury96 (AB368178), Eurychasma dicksonii
Eury05 (AB368177), Haptoglossa heterospora Drechsler (four sequences; AB253780,
AB253781, AB253782, and AB253783), Haptoglossa sp. (three sequences; AB253784,
AB253785, AB253786), Hylal operonospora parasitica (AY286223), Olpidiopsis
porphyrae (AB287419), Peronosclerospora sorghi (W. Weston & Uppal) C.G. Shaw
(AY286225), Peronospora manshurica (Naumov) H. Sydow (AY286222),
Peronospora tabacina D.B. Adam (AY286228), Phytophthora infestans (Montagne) de
Bary (U17009), Pythium acanthicum Drechsler (AY286219), and Thraustochytrium
aureum S. Goldstein (AF288091). The alignment was manually refined.

The molecular phylogenetic tree was generated with PAUP*4.0b10 software
(Swofford 2003). All gaps were treated as missing. A heuristic search procedure using
the tree bisection-reconnection (TBR) branch-swapping algorithm was performed to
find the optimal maximum likelihood (ML) tree topology. The transition/transversion
(ti/tv) ratio was estimated by maximizing the likelihood value for the neighbor-joining
(NJ) topology (Saitou & Nei 1987). The NJ topologies calculated with HKY85 model
(Hasegawa et al. 1985) were used as the initial topology for a heuristic search. The
bootstrap values were calculated based on 1000 bootstrap replicates of NJ method with
HKY85 model and 100 bootstrap replicates of ML method calculated from a heuristic
search procedure using the subtree pruning regrafting (SPR) branch-swapping
algorithm.

ML tree inferred from COII amino acid sequences was constructed using proml
program with “global rearrangements” option, in a PHYLIP version 3.65 package
(Felsenstein 1989). Jones-Taylor-Thornton (JTT) model (Jones et al. 1992) was used for
the analysis. The bootstrap values were calculated based on 1000 bootstrap replicates of
NJ method performed with protdist and neighbor programs, and 100 bootstrap replicates
of ML method performed with proml program in a PHYLIP version 3.65 package
(Felsenstein 1989).

Our sequences have the accession number AB363063 (SSU rRNA gene) and
AB363064 (cox2 gene). Alignments are also available in TreeBASE
(http://treebase.org/treebase/).

RESULTS

Description

Olpidiopsis bostrychiae S. Sekimoto, T. A. Klochkova, J. A. West, G.W. Beakes & D.
Honda sp. nov.

Thalli holocarpici, endobiotici; sporangia sphaerica, ellipsoidea, 20-55 µm longa ×
12-40 µm lata in cellulae ellipsoideis et 12-100 µm in diametro in cellulae sphaelicis,
cum uno (raro duobus) tubo emittenti protrudenti abs cortice algaceo ad maturitatem;
zoosporae ovoideae ad reniformes, 3.5-5.0 µm longae × 2.5-3.5 µm latae, in lateralibus
biflagellatae, maturae in zoosporangio; zoospora morphia ignota; sporae quiescentes
ignota; endoparasitus obligatus in Bostrychia spp., Stictosiphonia intricata
gametophyton, Porphyra spp. sporophyton, et interdum Heterosiphonia japonica
(Rhodophyceae).
Thalli holocarpic, endobiotic; sporangia spherical, ellipsoidal, 20-55 µm long × 12-40 µm wide in ellipsoid cells and 12-100 µm in diameter in spherical cells, with one (rarely two) discharge tube protruding from the algal cortex at maturity; zoospores ovoid to kidney shaped, 3.5-5.0 µm long × 2.5-3.5 µm wide, laterally biflagellate, maturing in a zoosporangium; zoospore morphism unknown; resting spores unknown; obligate endoparasite in *Bostrychia* spp., *Stictosiphonia intricata* gametophyte, *Porphyra* spp. sporophyte, and occasionally *Heterosiphonia japonica* (Rhodophyceae).

**HOLOTYPE:** TNS-F-17096 (Resin-embedded EM specimen), National Museum of Nature and Science (TNS). Type specimens are parasitic in *Bostrychia moritziana* 4314 (CCAP 1357/8).

**PARATYPES:** TNS-F-17097 & TNS-F-17098 (Resin-embedded EM specimen), National Museum of Nature and Science (TNS).

**TYPE LOCALITY:** Chenal d’Ampanarata, Belo sur Mer, Madagascar (20° 44.821’ S, 43° 59.654’ E).

**TYPE CULTURE:** CCAP 4055/1, Culture Collection of Algae and Protozoa, SAMS Research Service Ltd. Dunstaffnage Marine Laboratory, Dunbeg, Argyll, PA37 1QA, UK. Type culture is parasitic in *Bostrychia moritziana* 4314 (CCAP 1357/8).

**ETYMOLOGY:** Originally infects red algae *Bostrychia* spp.

GenBank accession numbers: AB363063 (SSU rRNA gene) and AB363064 (cox2 gene).

**Host species susceptibility to *Olpidiopsis bostrychiae* infection**

The pathogenic virulence of this Madagascan isolate of *Olpidiopsis* from *Bostrychia* tested against a wide range of potential red algal hosts has been summarized in Table 1. When it was first described it appeared that the isolate of *Olpidiopsis* appeared to be highly specific to members of this genus *Bostrychia* (see West *et al*. 2006 for detailed summary of the susceptibility results). *Stictosiphonia intricata* (Bory de Saint-Vincent)
P. C. Silva also showed minimal susceptibility to this parasite as well as the conchocelis (sporophyte) phase of Porphyra spp. from several distant localities (West et al. 2006 and Table 1). However, over the 3 years that this isolate has been in culture, it appears that its ability to infect the Porphyra conchocelis stage has attenuated whereas its virulence to B. moritziana 4314 has remained consistently high. Whilst this isolate of the parasite could successfully infect an isolate of Heterosiphonia japonica Yendo from Korea (Figs 13-16, 25, 31), it showed no virulence against isolates of the same host from Norway or other members of the same genus (Table 1). At times as many as 15-24 parasite thalli were observed in a single infected cell of H. japonica within 24 hours of inoculation with this parasite, but more often plants showed no signs of infection even after 10-14 days of dual culture with the parasite.

The infection by this parasite also appeared to be specific to certain cell-types. In particular the rhizoids and extended rhizoid-like lateral cells appeared susceptible to infection whereas the cells of the main axis and apex were rarely infected. This parasite required new host plant after consuming all suitable available host cells. After 3 years of laboratory culture the virulence of this isolate to H. japonica appears to have disappeared whereas its virulence to B. moritziana 4314 has remained for long term. Therefore, this parasite tends to generally prefer Bostrychia spp. as a host and this appears to be its principle defining characteristic.

Morphological observations of infection and parasite development

When healthy plants of Bostrychia moritziana 4314 were mixed pre-incubated with portions of thalli already infected with O. bostrychiae it usually took about 4-8 hours to initiate infection. Infection is started by zoospores of Olpidiopsis bostrychiae settling and encysting upon the surface of the host cell (Figs 1-4). At first, the settled zoospores (Fig. 1), showed a certain amount of amoeboid movement (Fig. 2) before encysting and becoming tightly attached to the host cell surface (Fig. 3). Germination of attached cysts takes place (Fig. 4). The thick host wall was then breached by a narrow penetration hypha (Fig. 5). After approximately 6 to 10 hours, more or less spherical parasite thalli could be observed inside the host cytoplasm under the light microscope (Fig. 6). Under the transmission electron microscope (TEM) a still uninucleate young thallus appeared unwalled, and contained abundant mitochondria (Fig. 7) and a single large vacuole but details of the host and parasite boundary could not be clearly resolved because of the
poor fixation at this stage.

Over the next 7 to 30 hours the spherical to ovoid Olpidiopsis thalli continued to expand within the infected host cells of Bostrychia spp (Figs 8, 9) until they reached dimensions of ca. 20-55 µm long × 12-40 µm wide inside. Thalli in another host, Heterosiphonia japonica appeared more variable in size ranging from 12 to 100 µm in diameter (Figs 13-16). In Bostrychia spp., Stictosiphonia intricata, and conchocelis stage of Porphyra spp. the infected cells did not swell, whereas in case of H. japonica, they often swelled 2.5-4 times from their original diameter, even with only 1 parasite thallus contained inside (Figs 13-16). It was not uncommon to observe multiple infections in cells of B. moritziana 4314, which typically contained between 1 to 6 (max. 8) parasite thalli of variable size. Similarly infected cells of H. japonica contained from 1 to 6(10) (max. 24) parasite thalli, of varying sizes indicating that multiple infections are taking place over a prolonged time-frame (Fig. 14).

During the expanding stages of parasite development the infected host cells usually appeared highly vacuolate often resulting in the parasite thallus being mostly surrounded by host vacuole. The parasite thallus was presumably still surrounded by a thin layer of host cytoplasm and very often, the parasite thallus inside was connected to the host cell periphery by fine cytoplasmic threads (Figs 13, 14). By the time the parasite thalli had fully expanded this stage the host cytoplasm had generally degenerated and appeared dense and disorganized (Figs 8-11).

There does appear to be certain of fixation-induced shrinkage of the host and parasite thallus (Fig. 9), which probably also accounts for the space between the cell wall and the host cytoplasm (Fig. 11). At this stage the parasite thallus had rather rounded nuclei and is densely packed with mitochondria and vesicles with large dense-body inclusions (DBV) (Fig. 10), which are similar to those found in the most oomycetes (Beakes 1989). It should be noted that two pairs of centrioles appeared at opposed poles of the nucleus in some nuclei (Fig. 12), suggesting that nuclear division may take place at this stage.

Approximately by 45 hours after initiation of infection, spore-formation was initiated (Figs 17-19). By this stage nuclear division of the parasite seemed to finish and the cell was full of scattered cytoplasmic units, consisting of a single nucleus and a zone of perinuclear mitochondria. These units will develop into spore initials (Figs 18, 19). Each spore-initial had a single nucleus, mitochondria with tubular cristae, kinetosome and peripheral vesicles, and the vesicles some of which contained dense-body
inclusions were around each spore initial (Figs 18, 19). Those dense-body inclusions were much smaller than ones found in the young spherical thalli (Figs 9-11). The vesicles which are closely associated with the perinuclear mitochondria appeared to be flimmer vesicles (fv) containing the proteinaceous flagellar mastigoneme hairs, (Fig. 19), which form early in zoosporangium development as in many other oomycete species such as *Saprolegnia* Kützing ex Warming (Heath & Greenwood 1971; Beakes 1994).

Continued thallus enlargement is associated with the appearance of centrally located vacuoles, that could be observed under both light and transmission electron microscopes (Figs 13, 15-18). As a result the cytoplasm became concentrated at the thallus periphery (Fig. 18). Although the overall quality of cytoplasmic preservation was not good at this stage, it was apparent that the mitochondria were still clustered around the nuclei and their associated kinetosomes (Fig. 19).

The transformation from thallus to immature zoosporangium was marked by the formation of a discharge tube which pushed through the thick host cell wall (Figs 17, 20-23). Very occasionally in infections of *B. moritziana*, *Olpidiopsis* thalli with two discharge tubes were observed (Fig. 21). The spore discharge tube of immature thalli contained many small vesicles with fibrillar contents inclusions that were concentrated in the discharge tube (Fig. 22; arrowheads in Fig. 23). In this particular tube there appeared to be an additional, inner wall layer which had trapped a dense layer of external membranous material (Fig. 23), similar to wall inclusion bodies known as lomasomes described in other oomycetes (L, Fig. 23) and which may be fixation-induced.

Fully differentiated zoosporangia (Fig. 25) have a dense, coarsely granular cytoplasm and completely lack the conspicuous large vacuoles observed at younger stages. No zoospore movement is observed within the sporangium until discharge takes place. A mature thallus containing almost fully differentiated zoospore initials, separated by a cleavage vesicle system containing dense granular contents which are still interconnected by fine strands of cytoplasm is shown in Fig. 24. There was no longer a close perinuclear cluster of mitochondria and the peripheral cytoplasm contained scattered DBV. However, no finger-print like inclusions within these vesicles were observed. Neither were there any axonemal profiles in the cleavage furrows at this stage, indicating flagellum formation probably does not take place until after cleavage is fully
The zoospores were liberated directly from the zoosporangium without the formation of a vesicular structure at the orifice of the sporangium (Fig. 25). Zoospores were ovoid to kidney shaped structures, approximately 3.5-5.0 µm long by 2.5-3.5 µm wide, with laterally inserted flagella (Figs 26, 27). Zoospore swimming behavior was fast and direct similar to that reported in many oomycete species with secondary-type zoospores (Dick 2001). The only zoospores that could observe under the electron microscopy were within zoosporangium and again the overall quality of cytoplasmic preservation was not particularly good (Figs 28, 29). Flagellar axonemal profiles are seen attached laterally to the zoospore and within the cleavage furrows (Figs 28, 29). A transitional helix structure was not observed but this could have been due to the immaturity of the spores, poor fixation or lack of suitable median sections through the flagellar base (Figs 28, 29). The zoospore contained a prominent nucleus, as well as mitochondria with tubular cristae, a prominent lipid body, dense-body vesicles and a number of spherical peripheral encystment-type vesicles (Figs 24, 28, 29). These have a structured periphery and electron dense cortex (Fig. 30). However, we did not observe a larger kinetosome-associated ‘K-body’ like structure, such have been observed in both O. porphyrae (Sekimoto et al. 2008a) and O. saprolegniae (A. Brown) M. Cornu (Bortnick et al. 1985).

Most thalli successfully liberated all of their contents and all that remained was the empty sporangia (Fig. 31), but in some sporangia not all zoospores escaped from the mature thalli and the remained spores encysted in situ (Figs 32-34). Unfortunately, these mature cysts did not fix well (Figs 32-34) but we could observe that the cysts contained single nucleus, mitochondria, dense body vesicles, kinetosomes, flimmer vesicles, and peripheral vesicles. The cyst had a cyst coat structure, which was a thin wall of the cyst (Figs 33, 34). There was no evidence of sexual reproduction occurring in this isolate.

**SSU rRNA gene molecular phylogeny**

Total genomic DNA of both infected and uninfected *Bostrychia* thalli were amplified with the eukaryote-universal primers *SR1* (Nakayama *et al.* 1996) and *18S13* (Honda *et al.* 1999). PCR products were produced from both infected and uninfected host tissue, suggesting that SSU rRNA gene regions of both host and parasite were amplified with this primer set. Both PCR products were TA cloned, and one type (host origin) or two
types (host and parasite origins) of PCR products in *Escherichia coli* plasmid DNA were found from uninfected and infected samples, respectively (Fig. 35). Two types of PCR products obtained from infected sample could not be distinguished when those PCR products were not digested by the restriction enzymes (See lanes 2 & 3 in Fig. 35) but they were clearly distinguished when they were digested (See lanes 5 & 6) and one of whose restriction pattern was agree with that from uninfected one (Lanes 4 & 5).

Four positive clones (showing restriction pattern like lane 6) were sequenced and totally, 1731 bp of the parasite SSU rRNA gene sequence was sequenced. Group I Intron-like long insertions were not observed in the SSU rRNA gene of this parasite, that was different from *Olpidiopsis porphyrae*, which contains four Group I Intron-like long insertions in that gene (Sekimoto et al. 2008a).

In the maximum likelihood (ML) tree inferred from SSU rRNA gene (Fig. 36), all oomycete species formed a monophyletic clade, which was supported by high bootstrap value [100%/100% of ML/NJ bootstrap (BS) values]. Although *O. bostrychae* formed a monophyletic clade with *O. porphyrae* with high statistical support (96%/98% of ML/NJ BS values), significantly branch length was quite long not short. The length was almost equal to one between *Hyaloperonospora parasitica* and *Plasmopara viticola* (in peronosporalean oomycetes) or the one between *Hyphochytrium catenoides* Karling and *Rhizidiomyces apophysatus* (hyphochytrids). Two *Olpidiopsis* species, oomycete marine crustacean parasite *Haliphthoros milfordensis* NJM0131, and abalone endoparasite NJM0034, marine crustacean parasite morphologically similar to *Haliphthoros* species, were branched before the main saprolegnian and peronosporalean lineages being supported by rather high bootstrap value (93%/70% of ML/NJ BS values), and also branched after a nematode parasitic oomycete *Haptoglossa* Drechsler (Beakes et al. 2006) and brown algal parasitic oomycete *Eurychasma dicksonii* (Küpper et al. 2006; Sekimoto et al. 2008b), whose branching was supported by high bootstrap value (94%/99% of ML/NJ BS values). The ML and NJ trees showed similar branching patterns (data not shown).

**COII amino acid molecular phylogeny**

Total genomic DNA of both infected and uninfected *Bostrychia* thalli was amplified with primers *peronosporomycete forward* and *peronosporomycete reverse* (Hudspeth et al. 2000). The PCR product was TA cloned and three clones were sequenced. All
sequences were identical and had a high similarity with other oomycete cox2 gene
sequences available in sequence databases. Thus, we concluded that this sequence
corresponds to the cox2 gene of *O. bostrychiae*. In total, 571 bp of the partial cox2 gene
sequence of *O. bostrychiae* was determined. All gapped sites were excluded and
translated 167 amino acids were used for analysis.

In the COII amino acid ML tree (Fig. 37), all oomycete species formed a
monophyletic clade, with very low bootstrap support (31%/18% of ML/NJ BS values,
not shown in the tree). *Olpidiopsis bostrychiae* branched after the monophyletic clade
including two *E. dicksonii* and seven *Haptoglossa* species but did not form a
monophyletic clade with other oomycete species. *Olpidiopsis porphyrae* formed a
monophyletic clade with three *Haliphthoros*. This result was different from the one of
SSU rRNA gene tree in which both *Olpidiopsis* species formed a robust monophyletic
clade but they were both branched before the main saprolegnian and peronosporalean
lineages in both trees. However, the position of all genera branched before the main
saprolegnian and peronosporalean lineages in the tree tended to be changed depending
on analysis settings and most nodes were not supported by bootstrap value. The ML and
NJ analyses yielded similar results (data not shown). The corresponding cox2 DNA
sequence trees were also comparable although the data set had a fairly low
transition/transversion (ti/tv) ratio (0.519647, data not shown).

**DISCUSSION**

**Identification of the Bostrychia parasite**

Because the genus *Olpidiopsis* shows very simple morphology, such as a spherical
unicellular endobiotic sporangium, species taxonomy of this genus is based upon
relatively few morphological features (shape, size of the thalli, and number of discharge
tubes) and host specificity (Sparrow 1960). Dick (2001) split up the genus and placed
*Olpidiopsis* species into 3 genera. He reserved the original genus *Olpidiopsis* for all
terrestrial and freshwater species and re-allocated the marine species to either *Pontisma*
H. E. Petersen for the red algal parasites, and *Sirolpidium* H. E. Petersen for the green
algal parasites. However, his re-classification scheme for the genus *Olpidiopsis* was not
based upon any ultrastructural and molecular data but was solely based on his own
speculative perspectives. Monophyly of the genus *Olpidiopsis* was questioned by Dick
(2001) and Sekimoto et al. (2008a) because marine Olpidiopsis species never formed resting spores whereas terrestrial and freshwater species, including the type species of the genus O. saprolegniae, did produce echinate resting oospores (Martin & Miller 1986c). Molecular phylogenetic studies on the genus Olpidiopsis including O. saprolegniae and other terrestrial/freshwater species is still needed to elucidate the phylogenetic relationship among oosporic/non-oosporic Olpidiopsis species. For this reason, it was decided at this stage not to adopt the revised nomenclatural scheme proposed by Dick (2001) when naming this Bostrychia parasite and instead we chose to continue to use the traditional classification concepts for the genus Olpidiopsis sensu lata as proposed by Sparrow (1960).

To date six non-oosporic Olpidiopsis species have been described as parasites of marine red algae [O. antithamnionis Whittick & South (Whittick & South 1972); O. dangeardii Feldmann & Feldmann (Feldmann & Feldmann 1967); O. feldmannii Aleem (Aleem 1952); O. magnusii Feldmann & Feldmann (Feldmann & Feldmann 1955); O. porphyrae (Sekimoto et al. 2008a)] and morphological comparison and host specificity of them are summarized in Table 2. Host specificity of the Bostrychia parasite using laboratory-controlled inoculation experiments described in West et al. (2006) and in this study showed that it infects four Bostrychia species [B. moritziana, B. radicans (Montagne), B. radicosa (Itono) J. A. West & G. C. Zuccarello, and B. tenella (J. V. Lamouroux) J. Agardh], Stictosiphonia intricata, the conchocelis stages of some Porphyra species [P. pulchella J. A. West & G. C. Zuccarello, P. tenera Kjellman, P. linearis Greville, and P. suborbiculata Kjellman], and occasionally Heterosiphonia japonica from Korea.

Morphologically the Bostrychia parasite most closely resembles O. antithamnionis, which has spherical or ellipsoidal thallus, produces mainly one, rarely more than one discharge tubes, and similar size of zoospore cells (Whittick & South 1972). However, host range of O. antithamnionis seems to be very restricted with infection reported only on Antithamnion floccosum (O.F. Müller) Kleen and not even on other species growing in close proximity, such as A. boreale (Gobi) Kjellman and Bonnemaisonia hamifera Hariot (Whittick & South 1972). In contrast, our Bostrychia parasite does not infect Antithamnion pectinatum (Montagne) Brauner, or A. sparsum Tokida but can infect Bostrychia spp., Stictosiphonia intricata, Porphyra spp., and occasionally Heterosiphonia japonica (West et al. 2006, this study).
As said by Whittick & South (1972), *Olpidiopsis antithamnionis* infected every cell of *Antithamnion flocossum* in culture, and such lack of immunity so that whole plant got infected also implied that the parasite was very specific to *A. flocossum*. This is similar to our observations of the *Bostrychia* parasite parasitizing in *Bostrychia* spp., but such widespread infection does not usually occur in other susceptible host algae, where only restricted infection occurs.

Interestingly, a Korean population of *H. japonica* occasionally sustained a cell-type (rhizoid) specific infection with this *Bostrychia* parasite, but other tested *Heterosiphonia* spp., including *H. japonica* from Norway, and *Dasysiphonia chejuensis* I. Lee & West and other *Dasya* spp. and were apparently resistant to infection. It is also noteworthy that the parasite has been remaining constant high susceptibility to *Bostrychia moritziana* 4314 from the year 2004, whereas susceptibility to *Porphyra* spp. and *H. japonica* (Korean isolate) was respectively reduced or completely lost. This implies a much higher specificity of this parasite to *Bostrychia* spp. than to any other potential hosts.

*Olpidiopsis dangeardii* infects *Radicilingua reptans* (Kylin) Papenfuss with which the *Bostrychia* parasite has not been tested (Feldmann & Feldmann 1967). However, sporangia of *O. dangeardii* are irregularly lobed and most commonly have two or three discharge tubes, and are thus closer morphologically to the genus *Petersenia* than to *Olpidiopsis* as also noted by Whittick & South (1972). *Olpidiopsis feldmanii* infects *Falkenbergia rufolanosa* (Harvey) Schmitz and *Trailliella intricata* Batters (Aleem 1952). But *O. feldmanii* zoospores are rather small (2-2.5 µm × 1.5-2 µm; Aleem 1952) and the *Bostrychia* parasite did not infect our *F. rufolanosa* culture (Table 1).

*Olpidiopsis magnusii* infects *Ceramium flabelligerum* J. Agardh but the *Bostrychia* parasite did not infect *C. japonicum* Okamura and two other unnamed *Ceramium* isolates that we tested (Feldmann & Feldmann 1955; West et al. 2006, this study). *Olpidiopsis porphyrae* infects both blade and conchoelis stages of *Porphyra* spp. and *Bangia* spp., but does not infect *Bostrychia moritziana* (Sekimoto et al. 2008a). The *Bostrychia* parasite does not infect blade stage of *Porphyra* (West et al. 2006, this study). There is rather limited morphological information and host specificity data for the described *Olpidiopsis* species infecting marine red algae available because those observations were mainly obtained from field samples. However, the overall morphology, host specificity data, and long-term stable virulence towards a specific algal host all support
our decision to treat the Bostrychia parasite as a new species of Olpidiopsis.

Transmission Electron Microscopy (TEM) of the Bostrychia parasite
The Olpidiopsis species on which TEM has been carried out are O. saprolegniae (Type species of the genus; Bortnick et al. 1985) and O. varians Shanor (Martin & Miller 1986a,b,c), both of which are the parasites of freshwater water molds, and marine red algal parasite O. porphyrae (Sekimoto et al. 2008a) but the zoospore ultrastructure was not investigated in O. varians. Both O. saprolegniae and O. porphyrae zoospores have subapically inserted flagella and single or double K-body-like structures. Whether both K-body-like structures are homologous is uncertain but they are different from O. bostrychiae zoospores, which have laterally inserted flagella and no K-body-like structure in their cytoplasm. The zoospores of O. porphyrae lacked extensive arrays of dense-body vesicles and peripheral encystment vesicles within the differentiated zoospores and that is different from O. bostrychiae zoospores. However, those features in O. porphyrae zoospores could be due to a fixation artifact as mentioned by Sekimoto et al. (2008a).

Many of the ultrastructural characters of the O. bostrychiae are shared with other oomycetes. Dense-body vesicles, the vesicles containing electron-dense inclusions, are often observed in the most oomycete lineages, including the order Saprolegniales, Leptomitales, and Peronosporales (Beakes 1989) but typical type of those vesicles is not observed in some marine species, O. porphyrae, Ectrogella perforans, Lagenisma coscinodisci, and Petersenia palmariae (Schneplf et al. 1978a; Raghukumar 1980a, b; Pueschel & van der Meer 1985; Sekimoto et al. 2008a). The zoospores of O. bostrychiae have a peripheral array of small electron-dense vesicles (Figs 23, 24) that appear to be homologous to the encystment vesicles described in many oomycetes (Beakes 1989).

There appear to be lomasomes at the tip of the sporangium discharge tube (Fig. 19). Lomasomes are aggregations of membranes in a matrix between the plasma membrane and cell wall that were named by Moore & McAlear (1961) and reviewed by Bracker (1967). Oomycete lomasomes have only been investigated in two saprolegnian species, Saprolegnia ferax (F. von P. Grunthuisen) F. T. Kützing and Dictyuchus sterile Coker (Heath & Greenwood 1970), and they suggested that lomasomes are produced from very active vesicle formation at the tip of the cytoplasm that can be recognized as a
region of active growth or reaction. However, Bracker (1967) suggested that they could be produced as a fixation artifact. We have no data how lomasomes are formed in the immature zoosporangium of this parasite but this observation would suggest that the tip of the discharge tube is very active at this stage in parasite development.

**Phylogeny of marine oomycete endoparasites**

Molecular phylogenetic analyses inferred from marine *Olpidiopsis* isolates did not form a monophyletic clade in the COII tree (Fig. 37) whereas the SSU rRNA gene tree showed their monophyly (Fig. 36). In COII tree branching the order among holocarpic oomycete endoparasites (including *Olpidiopsis*, *Haliphthoros* Vishniac, *Halodaphnea* Dick, and abalone endoparasite NJM0034) tended to be changed depending on tree settings, suggesting that their branching order is not well resolved using COII amino acids as a molecular marker. However, as a result our COII tree did not contradict our SSU rRNA gene tree, and our COII tree suggests that the two marine *Olpidiopsis* isolates had a significant genetic distance each other. The SSU rRNA gene tree showed a significant distance as well.

Recently, molecular phylogenetic approaches into basal oomycete lineages have been carried out as we did in this study (Beakes *et al.* 2006; Küpper *et al.* 2006; Hakariya *et al.* 2007; Sekimoto *et al.* 2007, 2008a,b). Both of our SSU rRNA gene and COII amino acid trees showed that the oomycetes seem to be divided into four groups (saprolegnian oomycetes, peronosporalean oomycetes, and basal holocarpic oomycete groups 1 & 2). Interestingly, basal genera which branched before the main saprolegnian and peronosporalean lineages are all marine holocarpic ones, excepting *Haptoglossa*, oomycete nematode parasites (Beakes *et al.* 2006). Although it is still unknown how *Haptoglossa* evolved, the oomycetes originating from marine environment have been discussed in some recent literature (Küpper *et al.* 2006; Cavalier-Smith & Chao 2006; Sekimoto *et al.* 2008a) and our data also indicated that the origin of oomycetes might well be from marine environment, and additionally, ancestral oomycetes are speculated to be marine endoparasites. In recent SSU rDNA molecular phylogenetic study for the overall phylogeny of stramenopiles, most closely related stramenopile organisms to monophyletic oomycetes were parasitoid nanoflagellate of marine diatoms *Pirsonia* spp., free-living heterotrophic flagellate *Developayella elegans* Tong, and hyphochytrids (Kühn *et al.* 2004; Cavalier-Smith & Chao 2006). Now only SSU rRNA gene sequences
of two freshwater hyphochytrids \textit{(Hyphochytrium catenoides} and \textit{Rhizidiomyces apophysatus}) are available but some hyphochytrids are marine saprophytes or parasites (Sparrow 1960; Dick 2001). Recent molecular studies of environmental DNA samples also found novel marine stramenopile lineages which located sister to the oomycetes (Massana \textit{et al.} 2002). Those results also suggest the marine origin of oomycetes. Interestingly, all basal genera have not been reported any sexual stages and resting oospores. Sparrow (1976) suggested that the marine environment is more stable than the terrestrial/freshwater environment so that they have little need for thick-walled resistant structure, such as oospores. Surprisingly, a sexual stage was found in \textit{Lagenisma coscinodiscii}, an oomycete endoparasite of marine diatoms (Schnepf \textit{et al.} 1978b), but was not oogamous, but involved the fusion of zoosporangiospores. Unfortunately no molecular work has been carried out on this species yet. It may be that many of these early branching marine parasitic oomycetes may have a non-oogamous sexual life cycle that has yet to be recognized. Many more combined ultrastructural and molecular studies for those oomycete endoparasites are needed to understand the early evolution and whole phylogenetic development of this important class.

ACKNOWLEDGEMENTS

We are grateful to Dr. Kath White, Vivian Thompson, and Tracy Davey (Electron Microscopy Research Services at University of Newcastle upon Tyne, UK) for their support in maintaining the electron microscope facilities. We are also grateful to Prof. Joseph L. Scott (College of William and Mary, USA) for giving us many suggestions for EM fixation of this marine red algal parasite, to Prof. Gwang Hoon Kim (Kongju National University, Korea) for many helpful suggestions, and to Prof. Jan Ruiness and Drs. Mi-Suk Hwan, Chris Schlech, and Sylvia Earle for providing some isolates used in the experiment.

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FIGURE LEGENDS

PLATE 1

Figs 1-16. Light and transmission electron micrographs of Olpidiopsis bostrychiae sp. nov. parasitic in the host algae Bostrychia moritziana 4314 (Figs 1-12) or Korean isolate of Heterosiphonia japonica (Figs 13-16), at the stages of early infection and vegetative thalli.

Figs 1-3. Amoeboid movement of the zoospore on the host cell surface (arrowheads). Scale bar is 4 µm.

Fig. 4. Empty spore cyst. Scale bar is 4 µm.

Fig. 5. Narrow germ tube (arrowhead) penetrates to the host cytoplasm through the host cell wall (HCW). Scale bar is 10 µm.

Fig. 6. Unwalled parasite thallus in the host cytoplasm (arrowhead). Scale bar is 10 µm.

Fig. 7. Transmission electron micrograph of very young unwalled parasite thallus (surrounded by arrowheads) in the host cytoplasm (HCy), showing nucleus (N) and mitochondria (m). Scale bar is 2 µm.

Fig. 8. Light micrograph of mature, walled parasite thalli (arrowheads) within the host cytoplasm (HCy). Scale bar is 20 µm.

Fig. 9. Transmission electron micrograph of mature, walled parasite thallus. Scale bar is 10 µm.

Fig. 10. High magnification of walled parasite thallus, showing multiple nuclei (N), mitochondria (m), and dense-body vesicles (DBV). Scale bar is 2 µm.

Fig. 11. The interface between the host (HCy) and the parasite (Cy) cytoplasm, showing the host cell wall (arrowheads). Scale bar is 1 µm.

Fig. 12. High magnification of the nucleus (N) of the walled parasite thallus. There appears to be the two pairs of polar centrioles (C) and golgi body (G). Scale bar is 1 µm.

Figs 13-14. Cytoplasmic threads connect parasite thalli with the periphery of the host cells (arrows). Arrowhead points to a parasite thallus which entered the host cell very recently, and larger developing sporangium lays next. Scale bars are 10 µm.

Figs 15-16. Despite of the presence of parasites inside, the host cells are still alive and their chloroplasts and vacuole (HV) are intact. Parasite thalli shown in the micrographs are over 10 h-old. Scale bars are 10 µm.
PLATE 2

Figs 17-23. Light and transmission electron micrographs of *Olpidiopsis bostrychiae* sp. nov. parasitic in the host alga *Bostrychia moritziana* 4314, at the stages of young sporangium and spore-initials.

**Fig. 17.** Light micrograph of young sporangium. The sporangium has two vacuoles (V) which are centrally-located, and a developing spore discharge tube (arrowheads). Host cytoplasm (HCy) was destroyed. Scale bar is 20 μm.

**Fig. 18.** Transmission electron micrograph of young sporangium, showing centrally-located vacuole (V). Scale bar is 5 μm.

**Fig. 19.** High magnification of the spore-initial in young sporangium. Mitochondria (m) are associated with flimmer vesicle (fv). Kinetosome (kt) and peripheral vesicles (arrowheads) are around nucleus (N). Scale bar is 1 μm.

**Fig. 20.** Transmission electron micrograph of young sporangium with spore discharge tube. Scale bar is 10 μm.

**Fig. 21.** High magnification of the tip of the spore discharge tube shown in Fig. 16. The vesicles (arrowheads) are rather densely located below the tip and a lomasome (L) is at the tip. Scale bar is 2 μm.

**Fig. 22.** Light micrograph of single narrow spore discharge tube (arrowhead). Scale bar is 10 μm.

**Fig. 23.** Light micrograph of double spore discharge tubes (arrowheads). Scale bar is 20 μm.

PLATE 3

Figs 24-34. Light and transmission electron micrographs of *Olpidiopsis bostrychiae* sp. nov. parasitic in the host algae *Bostrychia moritziana* 4314 (Figs 24, 26-30, 32-34) or Korean isolate of *Heterosiphonia japonica* (Figs 25, 31), at the stages of zoospores and empty sporangium.

**Fig. 24.** Transmission electron micrograph of immature zoospores showing a flagellum (F), kinetosome (kt), mitochondria (m) associated with the nucleus (N), dense body vesicle (DBV) and lipid body (LB). Scale bar is 2 μm.

**Fig. 25.** Time-lapse video of the release of zoospores from mature sporangium. Zoospores are released within 5 to 10 min, depending on the size of sporangium. Scale bar is 5 μm.

**Figs 26-27.** Light micrographs of swimming zoospore, showing the two flagella.
Fig. 28. Transmission electron micrograph of zoospore in the zoosporangium, showing mitochondria with tubular cristae (m), lipid body (LB), flagella (F) and peripheral vesicles (arrowheads). Scale bar is 5 μm.

Fig. 29. Transmission electron micrograph of zoospore in the zoosporangium, showing spindle-shaped nucleus (N), dense body vesicle (DBV) and peripheral vesicles (arrowheads). Scale bar is 1 μm.

Fig. 30. High magnification of the peripheral vesicle in the zoospore cytoplasm. Scale bar is 100 nm.

Fig. 31. Through-focus images of empty sporangium showing thick cell wall, long liberation tube and liberated zoospores. Scale bar is 20 μm.

Fig. 32. Transmission electron micrograph of the zoosporangium in which some cysts (arrowheads) are left. Scale bar is 5 μm.

Fig. 33. Transmission electron micrograph of cyst in the zoosporangium, showing nucleus (N), mitochondria (m), kinetosome (kt), peripheral vesicles (arrowheads) and cyst wall (CyW). Scale bar is 1 μm.

Fig. 34. Transmission electron micrograph of cyst in the zoosporangium, showing flimmer vesicles (fv) associated with mitochondria (m), dense body vesicle (DBV), peripheral vesicle (arrowheads) and cyst wall (CyW). Scale bar is 500 nm.

PLATE 4

Fig. 35. Confirmation of plasmid DNA containing the host (Bostrychia) or the parasite (Olpidiopsis) SSU rRNA gene regions by their restriction patterns obtained by a combined EcoRI and EcoRV digestion. Lane M, Size marker; Lanes 1 & 4, SSU rRNA region obtained from the uninfected host total genomic DNA; Lane 2 & 5, SSU rRNA region obtained from infected host total genomic DNA; Lane 3 & 6, SSU rRNA region obtained from infected host total genomic DNA. The inserted SSU rRNA gene regions in the plasmid DNA were amplified with the primer set T7 and SP6 (Lanes 1, 2, 3) and then digested by EcoRI and EcoRV (Lanes 4, 5, 6). Lanes 1 & 4, 2 & 5, and 3 & 6 are same clones but are undigested or digested, respectively. Lane 5 showed the same restriction pattern with lane 4 (host), suggesting the clone in lane 5 includes the host SSU rRNA gene regions. On the other hand, lane 6 showed the different restriction
pattern compared to lanes 4 & 5, suggesting the clone in lane 6 includes the parasite SSU rRNA gene regions.

**PLATE 5**

**Fig. 36.** Maximum likelihood tree (1403 sites) based on 38 SSU rRNA gene sequences of *Olpidiopsis bostrychiae* sp. nov. (in boldface), the organisms in the class Oomycetes, other basal stramenopiles, and two organisms in the Alveolata. Two alveolata organisms, *Amphidinium belauense* and *Prorocentrum minutum* were defined as an outgroup in the tree. Estimated ti/tv ratio is 1.574330. ML and NJ bootstrap values (100 and 2000 replicates, respectively) above 50% are indicated above the internodes.

**PLATE 6**

**Fig. 37.** Maximum likelihood tree (167 sites) based on 50 COII amino acid sequences of *Olpidiopsis bostrychiae* sp. nov. (in boldface), the organisms in the class Oomycetes, other basal stramenopiles, and two green and red algae (*Prototheca wickerhamii* and *Cyanidium caldarium*, respectively). *Prototheca wickerhamii* and C. caldarium were defined as an outgroup in the tree. Bootstrap values of ML (100 replicates) and NJ (2000 replicates), respectively, above 50% are indicated above the internodes.

**PLATE 7**

**Table 1.** List of algae tested for susceptibility to *Olpidiopsis bostrychiae* infection, their collection site or source.

**PLATE 8**

**Table 2.** Morphological and host specificity comparison between described non-oosporic *Olpidiopsis* species infecting marine red algae and *O. bostrychiae* sp. nov. Original table is from Whittick & South (1972).
Plate 1 (Figs 1-16).

Olpidiopsis bostrychiae: A new species of the endoparasitic oomycete that infects Bostrychia and other red algae.

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Plate 2 (Figs 17-23).

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Plate 4 (Fig. 35).
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Plate 5 (Fig. 36).

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