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ORIGINAL PAPER

Characterization of three novel species of Labyrinthulomycota isolated from ochre sea stars (*Pisaster ochraceus*)

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Abstract The Labyrinthulomycota (Stramenopiles) is an enigmatic group of saprobic protists that play an important role as marine decomposers, yet whose phylogenetic relationships and ecological roles remain to be clearly understood. We investigated whether members of this group were present on ochre sea stars (*Pisaster ochraceus*) showing symptoms of sea star wasting disease. Although largely decomposers, some members of the Labyrinthulomycota are also known to be opportunistic pathogens of animals such as abalone, clams and flatworms and cause severe wasting diseases in eelgrass populations worldwide. Three new isolates of Labyrinthulomycota were discovered from the tissues of *P. ochraceus* collected at Bamfield Marine Research Centre (48°83.6′N, 125°13.6′W) and Reed Point Marina (49°29.1′N, 122°88.3′W) in British Columbia. The new isolates were kept in culture for several months and characterized at the morphological level and with 18S rDNA sequences. Molecular phylogenetic analyses demonstrated that each of the three new isolates clustered within a different subclade of the Labyrinthulomycota: (1) *Oblongichytrium*, (2) *Aplanochytrium* and (3) an early diverging clade of environmental DNA sequences. These data enabled us to establish one new genus and three new species of Labyrinthulomycota: *Stellarchytrium dubum* gen. et sp. nov., *Oblongichytrium porteri* sp. nov. and *Aplanochytrium blankum* sp. nov. This is the first account of the Labyrinthulomycota isolated from the tissues of sea stars with a potential link to sea star wasting disease reported for *P. Ochraceus*.

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Introduction

The Labyrinthulomycota (Stramenopiles) are a mysterious and relatively understudied group of fungus-like marine protists (Leander et al. 2004). The group is distinguished by having an ectoplasmic net (branched extensions of the plasma membrane) produced by a novel organelle called a "bothrosome" (Raghukumar and Damare 2011). The Labyrinthulomycota is subdivided into three major subgroups—labyrinthulids, thraustochytrids and aplanochytrids—that are distinguished by molecular phylogenetic data (18S rDNA sequences) and differences in their cell shape and ectoplasmic net (Leander and Porter 2001). The phylogenetic relationships within the Labyrinthulomycota are poorly understood, largely because they are difficult to cultivate and have a high degree of morphological plasticity within a species that makes it comparatively common for an isolate to be incorrectly identified and named (Schärer et al. 2007; Leander and Porter 2001). Despite their ubiquity in marine environments, the ecological impacts of different Labyrinthulomycota are also poorly understood (Raghukumar 2002). However, their role in remineralisation is gaining more attention in recent times, as is their industrial use, as some species of Labyrinthulomycota are used in the commercial production of omega-3 fatty acids (Raghukumar and Damare 2011).

Although most species of Labyrinthulomycota are saprobes that feed on dead and decaying organic matter,

certain species are opportunistic pathogens of marine invertebrates and plants that cause severe wasting diseases in their hosts (Bower 1987a; Schärer et al. 2007; Ragan et al. 2000). For instance, quahog parasite X (QPX) is a thraustochytrid that causes massive mortality events in the quahog hard-shell clam *Mercenaria mercenaria* on the north-eastern coast of Canada and the USA (Anderson et al. 2003; Ragan et al. 2000). The organism is also found on healthy animals and marine sediments, suggesting an opportunistic nature (Burge et al. 2013). Another species of thraustochytrid, *Thraustochytrium caudivorum,* was found to be a pathogenic parasite of the free-living marine flatworm *Macrostomum lignano* and causes lesions beginning at the tail of flatworms that can lead to dissolution of the entire animal (Schärer et al. 2007). *Aplanochytrium haliotidis* acts as a facultative parasite and causes serious mortalities within abalone aquacultural systems in British Columbia and is transmissible without direct contact between individuals (Bower 1987a, b; Raghukumar 2002). Perhaps most dramatically, *Labyrinthula zosterae* causes a severe eelgrass wasting disease and is responsible for declines in eelgrass populations on both sides of the North Atlantic, the Mediterranean, and in the Pacific Northwest (Burge et al. 2013; Sullivan et al. 2013).

Based on previous reports of parasitism using invertebrates as hosts, we investigated whether members of the Labyrinthulomycota are found on individuals of *Pisaster ochraceus* (commonly known as ochre sea stars) whose population is currently being negatively affected by sea star wasting disease (Hewson et al. 2014). We isolated and cultured Labyrinthulomycota from the tissue of multiple sea stars in order to confirm their association with this host. Using morphological and molecular phylogenetic data, we demonstrated the presence of three new species of Labyrinthulomycota on ochre stars and established their molecular phylogenetic positions. We also discuss the potential relevance of these organisms to the current sea star wasting disease reported in *P. ochraceus*.

Materials and methods

Collection of *Pisaster ochraceus*

Sea star specimens were collected during September and October of 2014 and July 2015 from Bamfield Marine Research Station (Vancouver Island, British Columbia; 48°83.6′N, 125°13.6′W) and Reed Point Marina (Port Moody, British Columbia; 49°29.1′N, 122°88.3′W). Sea stars were collected from both the intertidal region and from docks at the marina. A total of 23 individual (*P. ochraceus*) sea stars were collected.

In vitro culture of the Labyrinthulomycota

The presence of the Labyrinthulomycota could not be determined by examination under a light microscope, which necessitated the isolation and culture of potential isolates. All sea star dermal tissue was externally rinsed with distilled water multiple times before being excised and placed onto culture plates to ensure that isolates came from the tissue of the stars. After rinsing the sea star dermal tissue, 1 cm^3 samples were excised and placed on culture plates containing Serum Seawater Agar (SSA, 1 % agar and 1 % horse serum) medium (Leander et al. 2004). The media were prepared using filtered, autoclaved seawater. In order to prevent bacterial growth, 100 mg ampicillin and 100 mg streptomycin were added to media. Cultures were kept at room temperature (20 °C) and transferred to new media plates every 2 weeks. This process was repeated with both symptomatic and asymptomatic sea stars. After cultures were established, subsamples were transferred to 2-keto-3-methylvalerate (KMV) media plates to determine optimal growth conditions (Porter 1989). These media are standard for thraustochytrids, which usually grow suboptimally, if at all, on SSA. KMV media contained 1 L seawater, 10 g agar, 0.1 g yeast extract, 0.1 g peptone, 1 g gelatin hydrolysate, 1 g glucose and <1 g GeO_2. Once the media were cooled, 200 mg ampicillin and 200 mg streptomycin were added, and the media were distributed into Petri dishes.

Morphological observations

We used DIC light microscopy to observe morphological features of the cultivated isolates of Labyrinthulomycota. We placed 1 cm^3 pieces of agar with growing cultures onto a slide with a few drops of autoclaved seawater and a coverslip. Images were taken on a Zeiss Axioplan 2 microscope using Leica FireCam and Zen software. Measurements of various features of the isolates, such as zoospores, vegetative cells (mature non-motile adult cells), sporangia (sacks that hold spores) and thickness of ectoplasmic nets, were taken using ImageJ software (Schneider et al. 2012).

DNA extraction, PCR and sequencing

Genomic DNA was isolated from the isolates using a standard protocol provided by the MasterPure complete DNA & RNA purification kit (Epicenter Biotechnologies, Madison, WI). PCR was performed on a BIO RAD MJ Mini Personal Thermal Cycler using illustra PuReTaq Ready-to-Go PCR beads (GE Healthcare), 23 µl of dH_20, 1 µl of genomic DNA and 1 µM of primer mix. An overlapping combination of three primer sets was used (Table 1). The PCR cycle consisted of an initial denaturing period (94 °C for

Table 1 Primers used in this study

Primer	Primer sequence (5′ → 3′)	Direction	Annealing temperature (°C)
NS1	GTAGTCATATGCTTGTCTC	Forward	46
NS4	CTTCCGTCAATTCCTTTAAG	Reverse	46
586	AGCCGCGGTAATTCCAGCT	Forward	55
1286	AACTAAGAACGGCCATGCAC	Reverse	55
891	GTCAGAGGTGAAATTCTTGG	Forward	50
1781	CCTTCCGCAGGTTCACCTAC	Reverse	50

4 min); 40 cycles of denaturing (94 °C for 30 s), annealing (temperatures varied for each primer set, Table 1); extension (72 °C for 2 min) and final extension period (72 °C for 10 min). PCR products were purified using ExoSAP-IT enzyme (USB, Affymetrix, Inc.). After PCR products were purified, they were sequenced in both directions using each primer set and Big Dye chemistry (Applied Biosystems).

Sequence alignment and molecular phylogenetic analysis

New SSU rDNA sequences from the isolates were edited using Sequencher 4.9 (Gene Codes Corporation, USA). The SSU rDNA sequences were screened and identified as novel using Basic Local Alignment Search Tool (BLAST) analysis and molecular phylogenetic analyses. Additional sequences used in the alignments were obtained from GenBank. ClustalX package (Genetics Computing Group, Madison, WI) was used to align the sequences, and fine manual alignment was done by eye using MacClade 4.08 (Maddison and Maddison 2001). A 63-taxon alignment was created with 852 base pairs included in the analysis. Jmodeltest 2 (Darriba et al. 2012) selected a general-time reversible (GTR) model of nucleotide substitution that incorporated invariable sites and a gamma-distributed rate variation among sites (GTR + I + G) under the Akaike information criterion (AIC) and AIC with correction (AICc). Maximum likelihood (ML) trees were constructed with RAxML BlackBox web server using a gamma model of rate heterogeneity (Stamatakis et al. 2008). Three alveolate species, *Euplotidium arenarium, Chromera velia and Heterocapsa triquetra,* were used as outgroups. Bayesian analysis of the 63-taxon dataset was performed using MrBayes 3.2.6 (Ronquist et al. 2012) under the GTR + I + G model (nst = 6; rates = invgamma), using default prior settings, and four Monte Carlo Markov chains (MCMC; default temperature = 0.2) in two independent runs for 10,000,000 generations. Trees were sampled every 100 generations after a burnin value of 2,500,000 generation (burnin = 25,000). Majority rule consensus trees were constructed from the 75,000 remaining trees. We consider ML bootstrap values of <40 and Bayesian posterior probabilities <0.80 low support. The final tree was edited in Adobe Illustrator (Adobe Illustrator CS6).

Results

Based on both molecular and morphological data, each of the isolates found in this study does not match any previously described species. This is also the first account of any Labyrinthulomycota isolated from this particular host, *Pisaster ochraceus*. The most closely related species to the organisms found in this study were uncultured clones from environmental DNA surveys, necessitating three novel species descriptions. Because one of the isolates placed within a strongly supported sister group to all other Labyrinthulomycota (containing sequences from other uncultured organisms as well), a new genus was needed in which to place these species.

Morphology of the three new isolates

Stellarchytrium dubum gen. et sp. nov

The following measurements were taken from isolates grown in pure culture on SSA medium for 2 months and then transferred onto a slide for observation. These isolates were found on a sea star collected from Reed Point Marina, in Port Moody, BC. Mature vegetative cells were round, immobile and ranged from 2.5 to 10 µm in diameter (Fig. 1b). Pigmentation was observed in some, but not all colonies (Fig. 1b, c). Ectoplasmic nets were thick and readily visible. On agar media, ectoplasmic nets grew as thin filamentous strands as small as 0.5 µm in diam. (Fig. 1d), and colonies formed clusters of ectoplasmic nets up to 200 µm wide (Fig. 1a–c). In liquid culture, ectoplasmic networks formed dense clumps that were different from filamentous strands observed in agar cultures (Fig. 1e). While the shape of the colonies differed between liquid and agar media, individual cell sizes were consistent throughout. Division through vegetative cytokinesis was observed on agar media. When sporangia were transferred onto slides and immersed in a few drops of seawater, swimming biflagellate zoospores that were ovoid in shape were observed within 30 min (Fig. 1f). The zoospores ranged from 2.0 to 2.3 µm in width and 3.5–4.0 µm in length. When transferred to KMV media, cells began to die after about 1 week, whereas they were easily kept alive and grew in thicker denser clumps on SSA media.

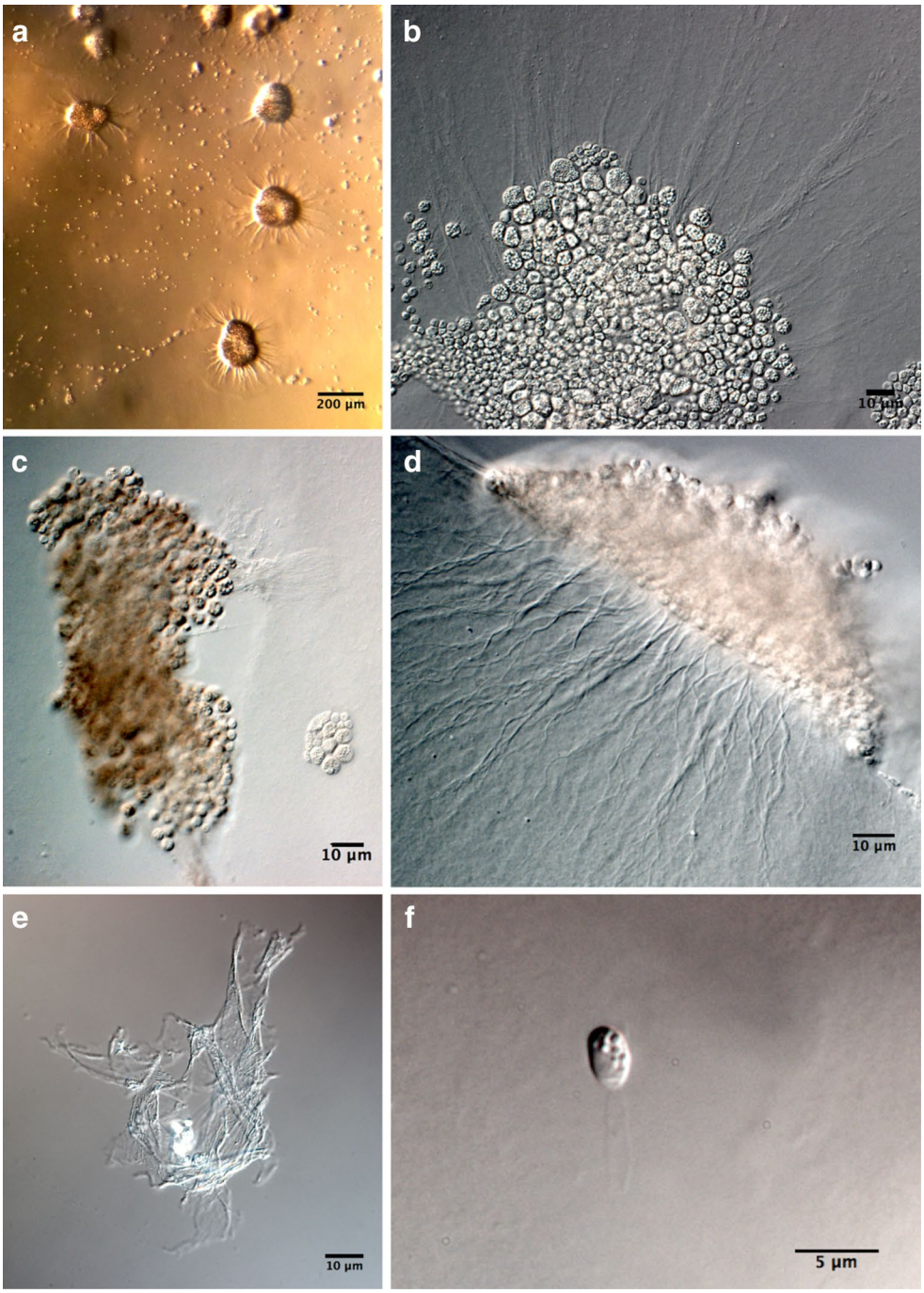


Fig. 1 DIC micrographs showing different morphological traits of *Stellarchytrium dubum* gen. et sp. nov. isolated from individuals of *Pisaster ochraceus* from Port Moody, BC in October 2014. **a** Colony growth pattern on agar. **b** Dense accumulations of vegetative cells grown on agar. **c** Accumulation of pigmented vegetative cells. **d** Vegetative cells with conspicuous ectoplasmic nets. **e** The form of colonies grown in liquid culture. **f** Biflagellated zoospore

Oblongichytrium porteri sp. nov

The following measurements were taken after 1 month in pure culture on SSA media. These isolates were found on 2 different individuals of *P. ochraceus* collected at Bamfield Marine Research Centre station and one individual sea star from Reed Point Marina. Mature cells of *O. porteri* were immobile and fusiform to orbicular in shape (Fig. 2b, c). Orbicular-shaped cells ranged from 2.3 to 7.8 µm (Fig. 2c), and fusiform cells measured 2.1–4.0 µm wide and 2.2–6.5 µm long (Fig. 2b). Some cells were also amoeboid in shape. No movement of mature cells was observed within

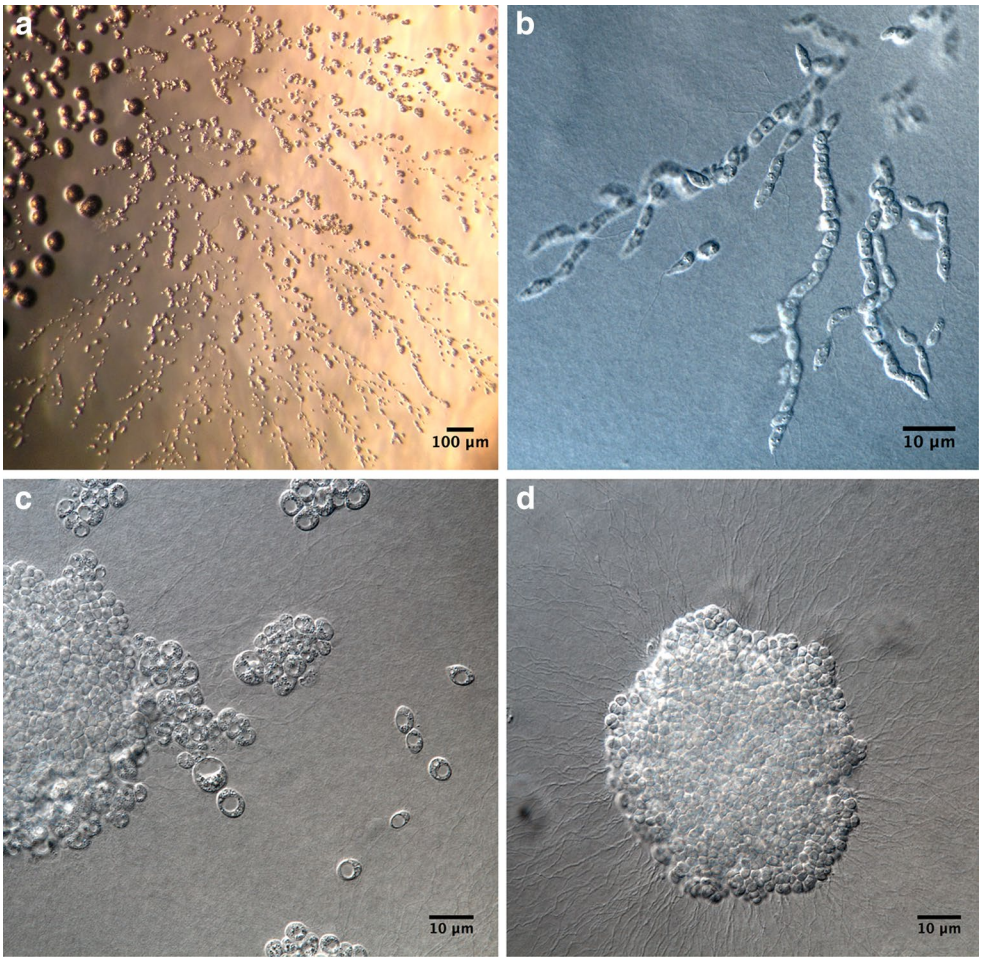


Fig. 2 DIC micrographs showing different morphological traits of *Oblongichytrium porteri* sp. nov. isolated from *Pisaster ochraceus* from Reed Point Marina in October 2014 and Bamfield Marine Sciences Centre in July 2015. **a** Pattern of colony growth on agar. **b** Higher magnification view showing individual vegetative cells connected to the ectoplasmic net. **c**, **d** Dense accumulations of cells grown on agar

or along ectoplasmic networks. Cells formed dense colonies that measured up to 60.3 µm in diam. (Fig. 2d). Additionally, colonies grew as branching rays (Fig. 2a). Most cells did not have any pigmentation, although some cells with light yellow colouring were observed. *O. porteri* contained nuclei located below the centre of the cells as well as large contractile vacuoles (Fig. 2b, c). The ectoplasmic nets of these organisms projected outward from the cells and were 1.0–1.3 µm in width. Between 10 and 55 spores were seen within observed sporangia. No free-swimming zoospores were ever observed. Colonies increased their number of cells through budding, which was observed on multiple occasions.

Aplanochytrium blankum sp. nov

The following measurements were taken after 1 month in pure culture on SSA media. Mature cells of *A. blankum* with fusiform, elliptical and amoeboid shapes were seen (Fig. 3b–d). These cells were not observed moving and ranged from 1.8 to 4.9 µm in width and 3.3–7.8 µm in length. On agar, the ectoplasmic nets of the cells grew as outward branching extensions that ranged from 0.5 to 1.2 µm in width (Fig. 3b). The colonies of cells were shaped as distinct rays that projected outwards from the tissue (Fig. 3a). 6–30 spores were observed within the sporangia, but no motile zoospores were seen (Fig. 3c, d). Centrally located nuclei and contractile vacuoles were observed.

Molecular phylogenetic analysis

Phylogenetic analyses of the 63-taxon alignment resulted in three well-supported clades: (1) a thraustochytrid clade, (2) a clade consisting of *Labyrinthula* and *Aplanochytrium* and (3) a novel environmental sequence clade

(Fig. 4). The environmental sequence clade contained the isolate from Reed Point Marina, namely *Stellarchytrium dubum* gen. et sp. nov.; therefore, we refer to this group as the "*Stellarchytrium* clade", which branches with 100 % bootstrap support as the sister group to all other Labyrinthulomycota sequences in the alignment (Fig. 4). The two isolates from the Bamfield Marine Science Centre were not closely related to each other and instead branched within two different subclades of the Labyrinthulomycota, namely *Oblongichytrium* and *Aplanochytrium* (Fig. 4). *Oblongichytrium porteri* sp. nov. branched within the *Oblongichytrium* clade with 98 % bootstrap support; *Aplanochytrium blankum* sp. nov. branched within the *Aplanochytrium/Labyrinthula* clade with 99 % bootstrap support and was the nearest sister species to an environmental DNA sequence (EF100337). The *Aplanochytrium/Labyrinthula* clade also contains two known pathogenic species of *Labyrinthula*, namely *L. zosterae* and *L. terrestris.* None of our isolates branched within the larger thraustochytrid clade.

Fig. 4 Maximum likelihood (ML) tree of 63 18S rDNA sequences inferred using RaXML. Bootstrap values are listed before Bayesian posterior probabilities above the branches. ML bootstrap values and Bayesian posterior probabilities <40 and 0.80, respectively, are not shown. Pathogenic species are denoted by *stars*; sequences from the three new species are highlighted in *black boxes*. *Scale bar* represents 0.1 mutations per site; each "/" corresponds to a *scale bar* omitted from the branch length for illustrative purposes

Discussion

The Labyrinthulomycota found in this study are novel for several reasons. Firstly, this is the first report of Labyrinthulomycota isolated from sea stars, specifically *P. ochraceus*. Secondly, it is unusual that these new species were cultured from living animal tissue, being that the Labyrinthulomycota consists mostly of saprobes (Leander et al. 2004). Lastly, molecular data suggest that all three species are previously undescribed and one represents the first observed species in a genus containing only sequences from environmental DNA surveys.

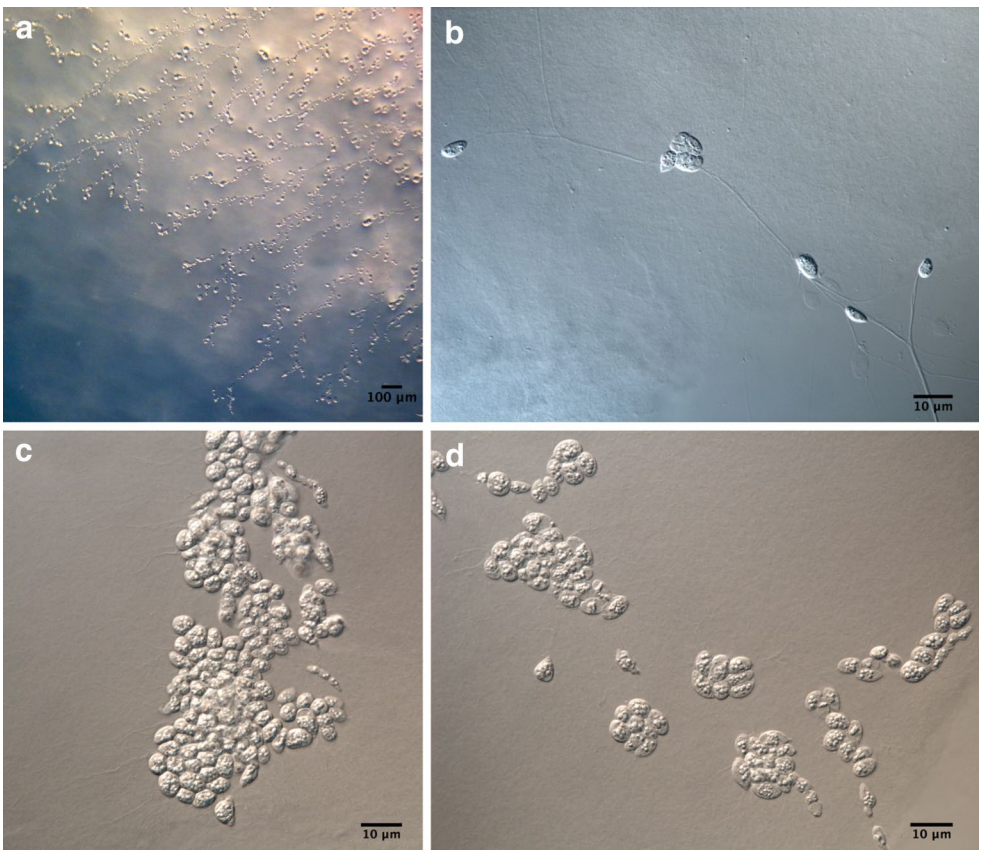
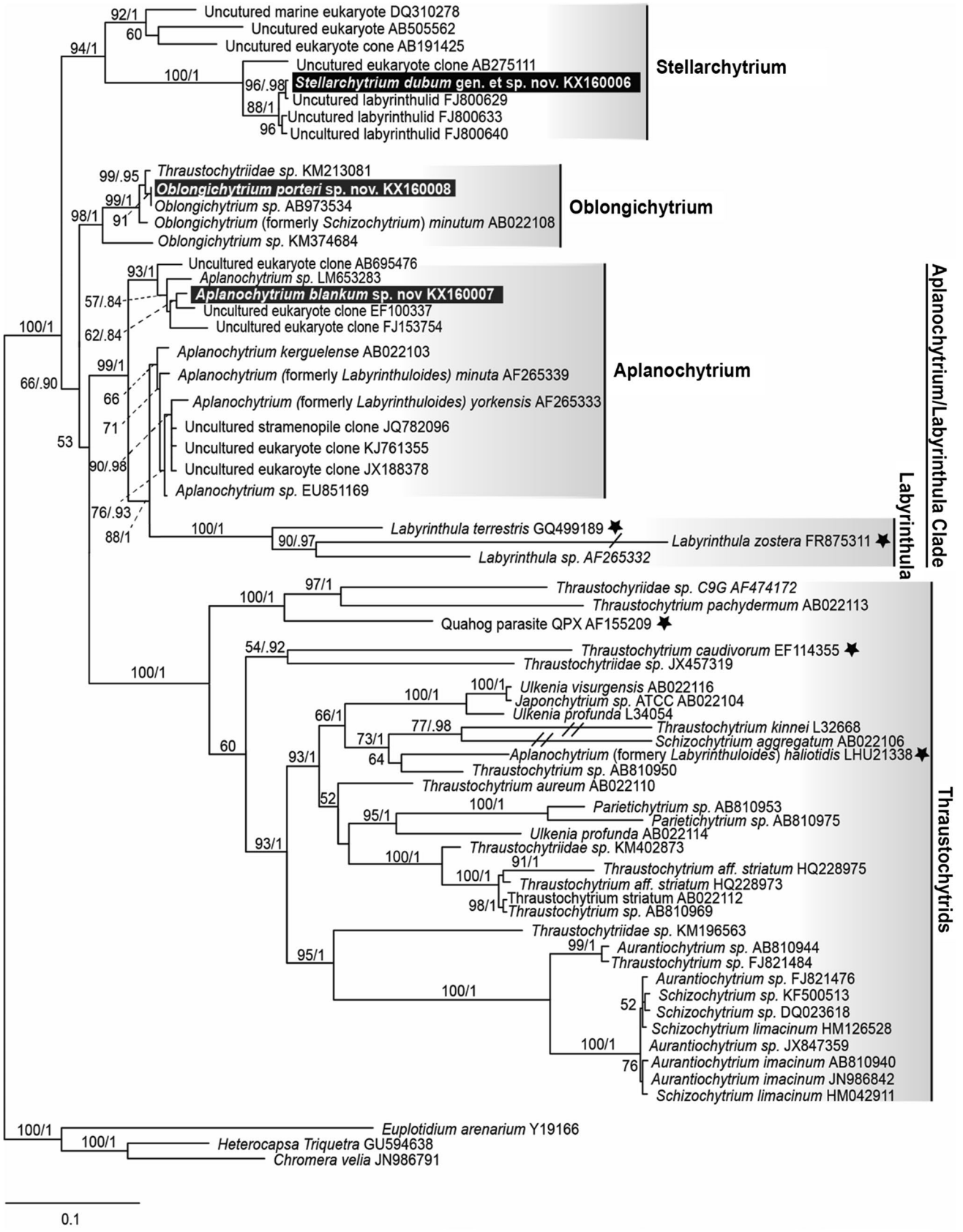


Fig. 3 DIC micrographs showing different morphological traits of *Aplanochytrium blankum* sp. nov. isolated from *Pisaster ochraceus* from Bamfield Marine Sciences Centre in July 2015. **a** Pattern of colony growth on agar. **b** Higher magnification view showing individual cells connected to the ectoplasmic net. **c**, **d** Dense accumulations of vegetative cells and sporangia grown on agar


Uncutured marine eukaryote DQ310278
Uncutured eukaryote AB505562
Uncutured eukaryote cone AB191425
Uncutured eukaryote clone AB275111
Stellarchytrium dubum gen. et sp. nov. KX160006
Uncutured labyrinthulid FJ800629
Uncutured labyrinthulid FJ800633
Uncultured labyrinthulid FJ800640
Stellarchytrium
Thraustochytriidae sp. KM213081
Oblongichytrium porteri sp. nov. KX160008
Oblongichytrium sp. AB973534
Oblongichytrium (formerly Schizochytrium) minutum AB022108
Oblongichytrium sp. KM374684
Oblongichytrium
Uncultured eukaryote clone AB695476
Aplanochytrium sp. LM653283
Aplanochytrium blankum sp. nov KX160007
Uncultured eukaryote clone EF100337
Uncultured eukaryote clone FJ153754
Aplanochytrium kerguelense AB022103
Aplanochytrium (formerly Labyrinthuloides) minuta AF265339
Aplanochytrium (formerly Labyrinthuloides) yorkensis AF265333
Uncultured stramenopile clone JQ782096
Uncultured eukaryote clone KJ761355
Uncultured eukaroyte clone JX188378
Aplanochytrium sp. EU851169
Aplanochytrium
Aplanochytrium/Labyrinthula Clade
Labyrinthula terrestris GQ499189
Labyrinthula zostera FR875311
Labyrinthula sp. AF265332
Labyrinthula
Thraustochyriidae sp. C9G AF474172
Thraustochytrium pachydermum AB022113
Quahog parasite QPX AF155209
Thraustochytrium caudivorum EF114355
Thraustochytriidae sp. JX457319
Ulkenia visurgensis AB022116
Japonchytrium sp. ATCC AB022104
Ulkenia profunda L34054
Thraustochytrium kinnei L32668
Schizochytrium aggregatum AB022106
Aplanochytrium (formery Labyrinthuloides) haliotidis LHU21338
Thraustochytrium sp. AB810950
Thraustochytrium aureum AB022110
Parietichytrium sp. AB810953
Parietichytrium sp. AB810975
Ulkenia profunda AB022114
Thraustochytriidae sp. KM402873
Thraustochytrium aff. striatum HQ228975
Thraustochytrium aff. striatum HQ228973
Thraustochytrium striatum AB022112
Thraustochytrium sp. AB810969
Thraustochytriidae sp. KM196563
Aurantiochytrium sp. AB810944
Thraustochytrium sp. FJ821484
Aurantiochytrium sp. FJ821476
Schizochytrium sp. KF500513
Schizochytrium sp. DQ023618
Schizochytrium limacinum HM126528
Aurantiochytrium sp. JX847359
Aurantiochytrium imacinum AB810940
Aurantiochytrium imacinum JN986842
Schizochytrium limacinum HM042911
Thraustochytrids
Euplotidium arenarium Y19166
Heterocapsa Triquetra GU594638
Chromera velia JN986791
0.1

Morphologically, *S. dubum* gen. et sp. nov. shares some characteristics with thraustochytrids such as globose sporangia and immobile adult cells (Leander et al. 2004). However, the molecular phylogenetic analysis showed that this species is more closely related to several environmental DNA sequences recovered from the coast of New York, deep-sea methane seeps in Japan, and a super-sulfidic anoxic fjord in Norway (Behnke et al. 2006; Takishita et al. 2007). This suggests that members of the *Stellarchytrium* clade can be found all over the planet. Further experiments to test the growth and survival abilities of *S. dubum* gen. et sp. nov. in various conditions will indicate the possible range of temperature, salinity and types of media under which this species can exist. The ecological role of this genus, and potential associations with hosts, remains to be explored.

Members of the genus *Oblongichytrium* are distinguished by the shape of the zoospores and the percentage of docosapentaenoic acid in total Polyunsaturated Fatty Acids (PUFA) (Yokoyoma and Honda 2007). Although the zoospores of *O. porteri* sp. nov. were not observed, the 18S rDNA sequence clustered strongly within the *Oblongichytrium* clade. The traits commonly used to describe clades within the Labyrinthulomycota do not correspond with the monophyletic groups inferred from molecular phylogenetic data, so the morphological criteria used for taxonomy in this group are currently undergoing substantial refinements (Honda et al. 1999; Yokoyoma and Honda 2007). Therefore, the interpretation of morphological traits within a molecular phylogenetic context is necessary to disentangle confusion in this group. The pale yellow colouring observed in this species, as well as the large colonies formed by continuous divisions, matches previous descriptions for *Oblongichytrium* (Yokoyoma and Honda 2007), and statistically there is strong support for the placement of this species within this genus. Of particular interest is that *O. porteri* was also found on more than one sea star at Bamfield as well as on a sea star from Reed Point Marina.

The three major groups within the *Aplanochytrium/Labyrinthula* clade represent two subclades of *Aplanochytrium* and a *Labyrinthula* clade, which is consistent with previous results (Collado-Mercado et al. 2010; Leander and Porter 2001; Tsui et al. 2009). *Aplanochytrium blankum* sp. nov. clusters within one of the *Aplanochytrium* clades also consisting of *Aplanochytrium* sp. (LM653283) and three environmental DNA sequences. The morphological features of *A. blankum* sp. nov. match descriptions of aplanochytrid growth on agar, as do the ectoplasmic nets that do not completely surround the cells (Fig. 3b) (Leander et al. 2004). Because the *Aplanochytrium/Labyrinthula* clade also contains known pathogens, *L. zosterae* and *L. terrestris*, the relatively close relationship of *A. blankum* sp. nov. to these species is of particular interest.

A notable observation is that the sea stars from which the Labyrinthulomycota were isolated all showed symptoms of sea star wasting disease. However, the nature of the relationship between the Labyrinthulomycota and their hosts remains unclear. Previous studies have found some members of the Labyrinthulomycota to be opportunistic pathogens that have been reported to survive on diverse kinds of media, such as *L. haliotidis*, which, in addition to using distantly related invertebrate species as hosts, can survive and thrive in salinities ranging from 10 to 40 %, temperatures ranging from 5 to 24 °C, and in sterile seawater (Bower 1987a; Bower 1987b; Burge et al. 2013). Our findings, combined with the ability of some species of Labyrinthulomycota to cause invertebrate epidemics when hosts are stressed, beg the question as to what is the relationship between these Labyrinthulomycota isolates and individuals of *P. ochraceus* showing symptoms of sea star wasting disease. We think further investigation into the connection between these isolates and symptomatic sea stars is warranted.

There is evidence to suggest that the primary pathogen of symptomatic sea stars is not eukaryotic or bacterial, but rather a densovirus (Hewson et al. 2014). Nevertheless, unanswered questions remain as to what has allowed a virus that has been present for over 70 years, and is also found in healthy animals, to presumably become lethal. Recent studies suggest that higher water temperatures increase the prevalence of the disease in sea stars (Eisenlord et al. 2015). Ecological diseases, however, are often the result of multiple species interactions that are difficult to disentangle, and future studies that examine the prevalence of Labyrinthulomycota on infected and non-infected sea stars are highly encouraged in order to investigate whether this group plays a role in sea star wasting disease (Hewson et al. 2014). Additionally, inoculation experiments of non-infected sea stars with the species described here could help elucidate whether infection induces symptoms of the disease or whether the Labyrinthulomycota are secondarily invading and taking advantage of already sick animals.

Here we report three new species of Labyrinthulomycota isolated from the dermal tissue of *P. ochraceus*. Because of the severity of the current epidemic and sea stars' role as keystone predators with a major impact on community structure (Uthicke et al. 2009), we hope this report stimulates studies that explore the ecological role of this enigmatic group of saprobes, the nature of their relationship to *P. ochraceus,* and whether there is any link to the current epidemic affecting sea stars.

Taxonomy

Stramenopiles Patterson (1989), amend Atl et al. 2005.
Labyrinthulomycota Whittaker 1969.

Stellarchytrium gen. nov. FioRito and Leander

Description: Mature cells were non-motile, either amber pigmented or colourless and with a single apical nucleus. Ectoplasmic nets radiating outwards from cell colonies growing on SSA media.
Type species: *Stellarchytrium dubum.*
Etymology: The generic name stems from the Latin noun stella which means "star" and refers to the host on which this genus was found.

Stellarchytrium dubum sp. nov. Fiorito and Leander (Fig. 1)

Description: The cell shape was globose, measuring 2.5–10.0 µm in diameter. Sporangia contained between 10 and 50 spores. Clumps of sporangia up to 200 µm wide. Biflagellate ovoid zoospore ranging from 2.0 to 2.3 µm wide and 3.5–4.0 µm long was observed swimming on slides. In liquid culture ectoplasmic networks form dense clumps that differ from filamentous strands observed on plates with agar. The shape of the colonies differed in liquid and agar media, but individual cell size was fairly consistent. Division through vegetative cytokinesis was observed on agar media. Sporangia grew in thicker, denser clumps on the SSA media compared to KMV media. Cells began to die after about 1 week on KMV media and were easily kept alive on SSA media through bimonthly transfers onto new media plates.
DNA sequence: SSU rRNA gene (GenBank accession KX160006).
Iconotype: Fig. 1b
Type locality: Reed Point Marina, British Columbia, Canada (49°29.1′N, 122°88.3′W).
Etymology: The specific epithet "dubum" refers to the surname of the first author's mother.
Type host: *Pisaster ochraceus* (Metazoa, Echinodermata, Asteroidea).
Location in host: Dermal tissue.

Oblongichytrium porteri sp. nov. Fiorito and Leander sp. nov. (Fig. 2)

Description: Mature, vegetative cells with fusiform to orbicular shapes were observed. No movement within or along ectoplasmic nets was seen. Orbicular cells ranged from 2.3 to 7.8 µm. Fusiform cells were 2.1–4.0 µm in width and 2.2–6.5 µm in length. Circular colonies of cells with diameters of up to 60.3 µm were seen. On media plates colonies grew as branching rays. Cells were mainly non-pigmented, but light yellow pigmentation was also observed. These organisms contained subcentrally located nuclei and contractile vacuoles. Ectoplasmic nets ranging from 1.0 to 1.3 µm in diameter were observed branching outwards from cells. Sporangia containing 10–55 spores were seen, but free-swimming spores were not. Increase in the number of cells in a colony through budding was frequently observed. Amoeboid-shaped cells were also observed.
DNA sequence: SSU rRNA gene (GenBank accession KX160008).
Iconotype: Fig. 2c.
Type locality: Bamfield Marine Sciences Centre, British Columbia, Canada (48°83.6′N, 125°13.6′W); Reed Point Marina, British Columbia, Canada (49°29.1′N, 122°88.3′W).
Etymology: Specific epithet "porteri" refers to the surname of the second author's PhD supervisor who spent his career studying this group.
Type host: *Pisaster ochraceus* (Metazoa, Echinodermata, Asteroidea).
Location in host: Dermal tissue.

Aplanochytrium blankum sp. nov. Fiorito and Leander (Fig. 3)

Description: Cells were fusiform, elliptical and amoeboid in shape. Mature cells ranged from 3.3 to 7.8 µm long and 1.8–4.9 µm wide. Ectoplasmic nets ranged from 0.5 to 1.2 µm wide and grew as branching extension from the cells. On agar, colonies grew as distinct rays projecting outwards. Sporangia with 6–30 spores observed. No free-swimming zoospores or pigmentation was seen. Nuclei were centrally located, and large contractile vacuoles were present.
DNA sequence: SSU rRNA gene (GenBank accession KX160007).
Iconotype: Fig. 3b.
Type locality: Bamfield Marine Sciences Centre, British Columbia, Canada (48°83.6′N, 125°13.6′W).
Etymology: Specific epithet "blankum" means white and refers to the lack of pigmentation in the cells and the name of the first author's sister/friend.
Type host: *Pisaster ochraceus* (Metazoa, Echinodermata, Asteroidea).
Location in host: Dermal tissue.

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Compliance with ethical standards

Ethical approval All applicable international, national and institutional guidelines for the care and use of animals were followed. All procedures performed in studies involving animals were in accordance with the ethical standards of the Institution University of British Columbia, Vancouver and Bam field where the studies were conducted.

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