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Fungal Infection of Mantis Shrimp (*Oratosquilla oratoria*) Caused by Two Anamorphic Fungi Found in Japan

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Abstract Two fungal pathogens of the mantis shrimp (*Oratosquilla oratoria*) in Yamaguchi and Aichi Prefectures, Japan are described as the new species *Plectosporium oratosquillae* and *Acremonium* sp. (a member of the *Emericellopsis* marine clade). Both fungi infect the gills of the mantis shrimp, which become brown or black due to melanization. The former species is characterized by its slow growth on artificial seawater yeast extract peptone glucose (PYGS) agar, pale yellow to pale orange or grayish yellow colonies, short cylindrical solitary phialides with a wavy tip, and one-celled ellipsoidal conidia. Although lacking the two-celled conidia demonstrated by the type species *Plectosporium tabacinum*, the taxonomic placement of the new species was confirmed by DNA sequence analysis of the internal transcribed spacer (ITS) region of ribosomal DNA (ITS1, 5.8S rDNA and ITS2). *Acremonium* sp., the other causal pathogen, differs from *P. oratosquillae* by its fast growth on PYGS agar, pale orange to salmon-colored colonies, long, slender conidiophores consisting of solitary phialides with tips lacking an undulate outline, and typically cylindrical conidia. Analysis of ITS and β-tubulin gene sequences placed this fungus within the phylogenetically distinct *Emericellopsis* (anam. *Acremonium*) marine clade. Various physiological characteristics of both pathogens were also investigated. This is the first report of a fungal infection found on the mantis shrimp in Japan.

Keywords *Acremonium* sp. · Fungal infection · Mantis shrimp · *Oratosquilla oratoria* · *Plectosporium oratosquillae*

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Introduction

The mantis shrimp (*Oratosquilla oratoria*) is an economically important and delicious culinary crustacean species. One of the famous Japanese 'sushi dishes' is made from the meat of the mantis shrimp. This shrimp is found on mud in the coastal areas of Japan and is the most dominant species, occupying

21.5% by biomass of the 20 most abundant species. Production of the shrimp met with a decline from 1991 to 1999, and continues to decrease due to environmental variations [1, 2] and other harmful factors.

In marine environments, some genera of pathogenic anamorphic fungi cause serious diseases of various aquatic animals. In crustaceans, *Fusarium* species cause black gill disease in the kuruma prawn (*Penaeus japonicus*) in Japan [3–7], lobster (*Homarus americanus*) in the USA [8] and tiger shrimp (*Penaeus monodon*) cultured in Vietnam [9]. Other anamorphic fungi are also pathogenic to crayfish [10], red sea bream [11], ayu fry [12], and young striped jack fish [13]. However, there are no reports of fungal infections in mantis shrimps. Consequently, we attempted to isolate the fungal pathogens from wild mantis shrimp, collected from April 2005 to December 2006 in Yamaguchi and Aichi Prefectures, Japan and describe here the identification of the isolates which infected the gills of this shrimp.

Materials and Methods

Mantis Shrimp Samples

Mantis shrimp of 17–30 g in body weight were sampled monthly from April 2005 to December 2006 by trawl net in Yamaguchi and Aichi Prefectures, along the Pacific side of central Japan. For each collection, there were 4–23 mantis shrimp with a fungal infection resulting in gill lesions (Figs. 1, 2).

Histological Findings

Shrimp tissues infected with fungi were fixed in 10% phosphate buffered formalin solution. The gills, muscle, and intestine specimens were embedded in paraffin, sectioned at 3–4 μm, and stained using the periodic acid Schiff (PAS).

Isolation of Fungi

Infected gills were washed three times in sterile physiological saline (0.85% NaCl) and inoculated on plates with artificial seawater yeast extract peptone glucose (PYGS) agar [0.125% Bacto peptone, 0.125% Bacto yeast extract, 0.3% glucose, 1.2% Difco agar, and 3.8% artificial sea salt (Aqua-Ocean®, Japan Pet Drugs, Tokyo)]. Ampicillin and streptomycin sulphate (500 μg ml^{-1}) were added to the medium to inhibit bacterial growth. Plates were incubated for 2–4 days at 25°C and then subcultured onto fresh PYGS agar plates. The single spore culture method of Ho and Ko [14] was applied to obtain pure isolates. The isolates were maintained at 25°C on PYGS agar for subsequent experiments.

Identification of Fungi

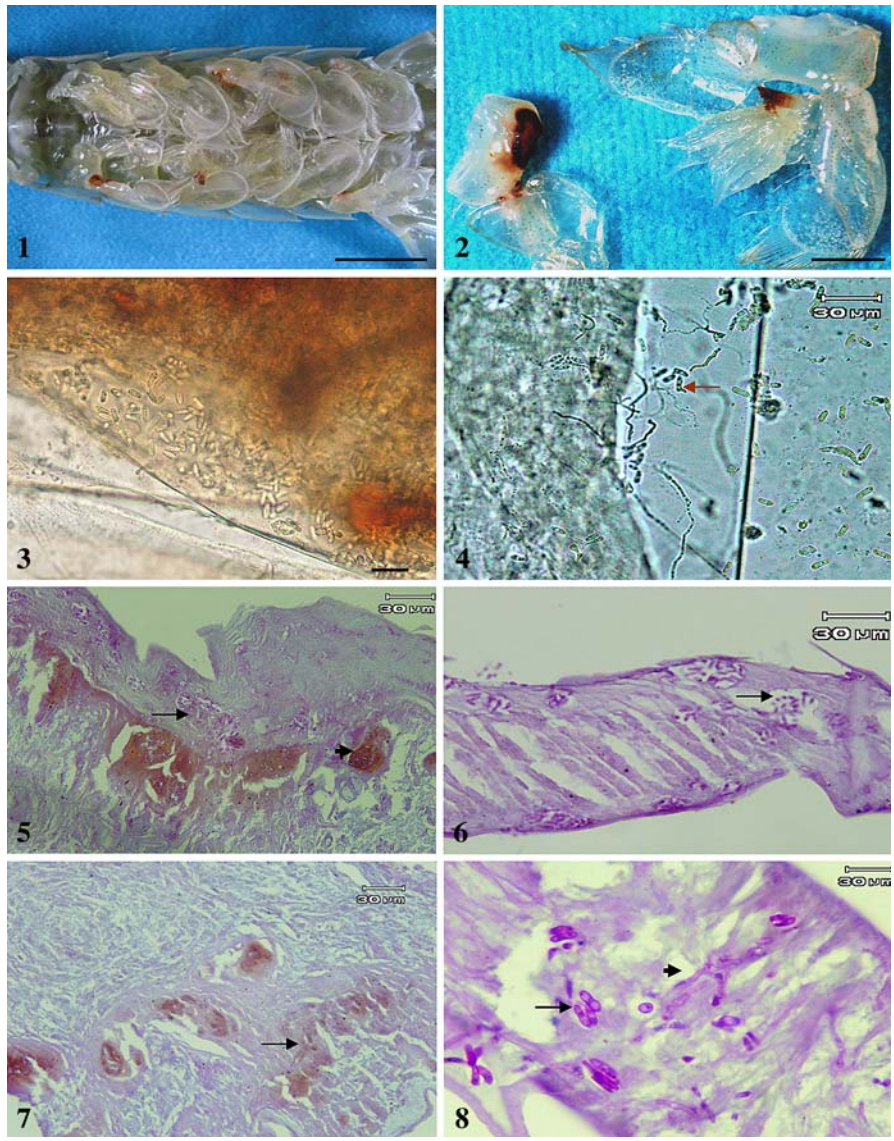
The strains studied are listed in Tables 1, 2. Reference strains are named according to their original designation in culture collections.

Morphology

Morphological characterizations of the isolates were made from colonies grown on PYGS agar, potato-dextrose agar (PDA, Nissui Phamaceutical Co. Ltd., Tokyo, Japan), potato-carrot agar (PCA), and Sabroud glucose agar (SGA, Nissui) after incubation at 20, 25, and 37°C. Slide cultures were also used to examine microscopic features. For the observation of conidial structures on slides, materials were stained with cotton blue in lactic acid or B solution (Fungal Flora Y Co., Ltd., Japan, commercial product for fungal observations under a fluorescent microscope). Capitalized color names and references in the descriptions are those of Kornerup and Wanscher [15]. For scanning electron microscope (SEM) observations, blocks of agar (2–5 mm^2) were cut from sporulating colonies of the isolates and fixed in 2% glutaraldehyde in 0.1 M phosphate buffer and 1% osmium tetroxide, dehydrated in a graded ethanol series, then critical-point dried (JEOL JFD-310). Specimens were mounted and sputter-coated with silver (JFC-1600 Auto Fine Coaster) and examined using SEM (JEOL JSM-6380 LV, Japan).

DNA Analysis and Sequencing

DNA was prepared using Gentorukun (Takara Bio Inc., Ltd., Otsu, Japan) from approximately 100-μl volume of fungal mass cultured at 25°C for 14 days on PDA slants. The ITS region (ITS1, 5.8S rDNA and ITS2) and β-tubulin gene were sequenced directly from PCR products using the primer pairs of ITS1 and ITS4 [16],



Figs. 1-8 Mantis shrimp with naturally fungal infection. **Fig. 1** Gills showed brown discoloration, bar = 2 cm; **Fig. 2** Gills disappeared due to fungal infection, bar = 1 cm; **Fig. 3** Numerous conidia of fungal group B inside the gill lamella, bar = 10 µm; **Fig. 4** Hyphae and conidial germination (arrow) of fungal group A inside the gill lamella; **Fig. 5** Hyphae and conidia (arrow) and fungal hyphae of group B were encapsulated (arrow head) at base of gill, PAS; **Fig. 6** Conidia of group B invaded in the gill lamella, PAS; **Fig. 7** Hyphae of fungal group A were encapsulated (arrow) at base of gill, PAS; **Fig. 8** Numerous hyphae (arrow head) and conidia (arrow) of fungal group A invaded in the gill lamella, PAS

and Bt2a and Bt2b [17], respectively. PCR products were sequenced using the BigDye Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Foster City, CA, USA) and the ABI PRISM 3130 Genetic Analyzer (Applied Biosystems), according to the manufacturer's instructions.

Molecular Phylogenetic Analysis

DNA sequences were edited using ATGC ver. 4 sequence assembly software (Genetyx Co., Ltd., Tokyo, Japan), and alignment of the sequences was performed using ClustalX software [18]. For neighbor-joining (NJ) analysis [19], the distances between sequences were calculated using Kimura's two-parameter model [20]. A bootstrap was conducted with 1,000 replications [21].

Physiology

Effect of Culture Media on Mycelial Growth of Isolates

Mycelial growth of the isolates was evaluated on seven media: PYGS agar, PDA, oatmeal agar (OA, Difco Laboratories, Detroit, MI, USA), SGA, cornmeal agar

Table 1 List of isolates from diseased mantis shrimp and the representative strains in this study (isolate group A)

Species, strain No.	Accession No. ITS	Substrate	Location	Date of isolation
Plectosporium sp.				
NJM 0573	AB425973[a]	Mantis shrimp (*Oratosquilla oratoria*)	Yamaguchi, Japan	Dec. 2005
NJM 0662	AB425974[a]	Mantis shrimp (*O. oratoria*)	Yamaguchi, Japan	Apr. 2006
NJM 0665	AB425975[a]	Mantis shrimp (*O. oratoria*)	Yamaguchi, Japan	May 2006
NJM 0667	AB425976[a]	Mantis shrimp (*O. oratoria*)	Aichi, Japan	Apr. 2006
NJM 0678	AB425977[a]	Mantis shrimp (*O. oratoria*)	Aichi, Japan	Sep. 2006
DAR76524	EU480709[b]			
DAR76526	EU480705[b]			
DAR76527	EU480691[b]			
RM1-12	DQ993622[c]	Marine sponge (*Suberites zeteki*)		
142C	EU480698[b]			
Plectosporium alismatis (Oudem.) W.M. Pitt, W. Gams & U. Braun				
CBS 113363 (DR76493)	AY572016[b]	Leaf spost (*Alisma plantago-aquatica*)	The Netherlands	
Gams 1B	AY572020[d]			
Gams 2A	AY572015[d]			
RH 01	AY258151[d]			
RH 62	AY258150[d]			
P. delsorboi Antignani & W. Gams				
CBS 116708[e]	DQ825986[b]	Leaf spost (*Curcuma alismatifola*)	Italy	
P. tabacinum (J.F.H. Beyma) M.E. Palm, W. Gams & Nirenberg				
NBRC 9985	AB425978[a]	Violet (*Vio odorata*)	Egypt	
NBRC 30005	AB425979[a]	River bottom mud		
NRRL 20430	AF176952[d]			
RH 126	AF258149[d]			
00017	AJ246154[d]			
380408	AJ492873[d]			
(none number)	DQ493934[c]			
Out group				
Verticillium nigrescens				
IMI 044575	AJ292440			
UHMH 6687	AF108473			
V. albo-atrum				
ATCC 44943	X60705			

[a] This study; [b] Ash et al. [23]; [c] Wang et al. [24] ; [d] Pitt et al. [26]; [e] Zare et al. [25]

(CMA, Nissui), malt extract agar (MEA, 3% Fluka malt extract, 0.5% Bacto peptone, 1.5% Difco agar), and mycosel agar (MSA, Eiken Chemicals Co. Ltd., Tokyo, Japan). Plates containing each of the seven media were inoculated according to Kim et al. [22], as follows: about 5-mm diameter agar plugs were removed with a sterile cork borer No. 2 from the leading edges of pre-cultured colonies, and one such plug was placed in the center of each 90-mm Petri dish containing a medium. The colony diameter in each plate was measured after incubation for 7 or 15 days at 25°C.

Effect of Temperature on Mycelial Growth of Isolates

The effect of temperature on mycelial growth was evaluated on PYGS agar. Inoculated plates, as described above, were incubated at 5, 10, 15, 20, 25, 30, 35, and 40°C. The colony diameter on each plate was measured after incubation for 8 or 15 days.

Table 2 List of isolates from diseased mantis shrimp and the representative strains in this study (isolate group B)

Species, strain No.	Accession No. of GenBank		Substrate	Location	Date of isolation
	ITS	β-tubulin			
Acremonium sp.					
NJM 0567	AB425971[a]	AB436949[a]	Mantis shrimp (*O. oratoria*)	Yamaguchi, Japan	May 2005
NJM 0672	AB425972[a]	AB436950[a]		Aichi, Japan	Sep. 2006
A. fuci Summerbell, Zuccaro & W. Gams					
CBS 112868	AY632653[b]	AY632690[b]	*Fucus serratus*, leaf	Germany	
A. potronii Vuill.					
CBS 378.70F	AY632655[b]	AY632691[b]	Salty soil	France	
A. tunakii					
CBS 111360	AY632654[b]	AY632689[b]			
Emericellopsis donezkii Beliakova					
CBS 489.71	AY632658[b]	AY632674[b]	Water (river)	Ukraine	
E. humicola (Cain). C. Gilman					
CBS 180.56	AY632659[b]	AY632675[b]	Peat soil	Canada	
E. glabra (J.F.H. Beyma) Backus & Orpurt					
CBS 119.40	AY632657[b]	AY63673[b]	Soil	Netherlands	
E. maritima Beljakova					
CBS 491.71	AY632670[b]	AY632686[b]	Coastal seawater	Ukraine	
E. microspora Backus & W. Gams					
CBS 380.62	AY632663[b]	AY632679[b]	Wet prairie soil	USA	
E. minima Stolk					
CBS 871.68	AY632660[b]	AY632676[b]	Wheat field soil	Germany	
CBS 111361	AY632661[b]	AY632677[b]	Brown algae (*Fucus serratus*)	Germany	
CBS 190.55	AY632669[b]	AY632685[b]	Mangrove soil	Mozambique	
E. pallida Beljakova					
CBS 624.73	AY632667[b]	AY632683[b]	Organic soil	Canada	
E. robusta Emden & W. Gams					
CBS 489.73	AY632664[b]	AY632680[b]	Agriculture soil	Netherlands	
E. salmosynnemata Grosklags & Swift					
CBS 382.62	AY632666[b]	AY632682[b]	Soil	Belgium	
E. stolkiae D.E. Dacidson & M. Christensen					
CBS 159.71	AY632668[b]	AY632684[b]	Mud in saline lake	USA	
E. synnematicola P.N. Mathur & Thirumalachar					
CBS 176.60	AY632665[b]	AY632681[b]	Soil	India	
E. terricola J.F.H. Beyma					
CBS 229.59	AY632662[b]	AY632678[b]	Dried river mud	Netherlands	
Stanjeminium frisellum W. Gams, Schroers & M. Christensen					
CBS 655.79	AY632671[b]	AY632687[b]	Soil from grassland	USA	
S. ochroroseum W. Gams & M. Christensen					
CBS656.79	AY632672[b]	AY632688[b]	Soil from grassland	USA	
Out group					
Bionectria aureofulvella					
CBS 195.93	AF358226[b]	AF358181[b]			

Table 2 continued

Species, strain No.	Accession No. of GenBank		Substrate	Location	Date of isolation
	ITS	β-tubulin			
B. oblongispora					
CBS 100258	AF358248[b]	AF358169[b]			
B. samuelsii					
CBS 699.97	AF358236[b]	AF358190[b]			

[a] This study; [b] Zuccaro et al. [27]

Effect of Concentration of Artificial Seawater on Mycelial Growth of Isolates in Group A

To examine the effect of the concentration of artificial seawater (ASW) on mycelial growth of the isolates (*Plectosporium oratosquillae*, see Results), the saline component of PYGS agar was amended with ASW at various concentrations (v/v): 100, 75, 50, 25, 12.5, and 0%. The salinity of ASW was prepared as 38‰. Inoculated plates, as described above, were incubated at 25°C. The colony diameter in each plate was measured after incubation for 15 days.

Effect of pH on Mycelial Growth of Isolates

The effect of pH on mycelial growth was evaluated in sterile PYGS broth, in which the pH was adjusted to a predetermined level by the addition of 1N solution of HCl or NaOH and filtrated through a cellulose aseptic filter (0.45-µm pore size; Toyo Roshi Kaisha Ltd., Tokyo, Japan) under aseptic conditions. The pH of the medium was adjusted to 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and 12.0. Each medium treatment (5 ml) was poured into a sterile test tube. As with the agar plates, inoculated test tubes were incubated at 25°C. The mycelial growth in each test tube was evaluated by visual comparison with a PYGS broth control (pH 7.0). The results from pH manipulation were recorded as: (0) no growth; (1) weak growth; (2) moderate growth; and (3) abundant growth. The inoculations that did not grow at the lowest and highest pH levels were washed in sterile distilled water and then placed onto fresh PYGS agar to determine whether the fungus was still viable.

Measurement of Growth Experiments

All experiments were carried out with three replicates per treatment. The colony diameter on each plate was measured by a vernier caliper after appropriate incubation. The radial growth rate of each isolate was calculated to give the final diameter value.

Results

Histopathology

The external clinical symptoms of a mantis shrimp with fungal infection were brown or black discoloration due to melanization and gill disappearance due to deliquescence. Hyphae with septa and conidia were observed in the infected gills under a microscope (Figs. 3, 4). The results of histological examination showed that fungal elements were present in the gills (Figs. 5, 6). Fungal hyphae were encapsulated in base of gills (Figs. 7, 8).

Mycology

Phylogenetic Analysis

Two groups of fungal strains were isolated coincidentally from mantis shrimp samples: Group A contained five isolates, NJM 0573, 0662, 0665, 0667, and 0678, which were easy to recognize in culture by their slow growth on all media, and group B contained two isolates, NJM 0567 and 0672, differing from A by their fast growth and an *Acremonium*-like colony appearance. The ITS sequences for all the seven NJM isolates, and the β-tubulin gene of the two NJM isolates in group B were determined.

The sequences of the ITS region on the five NJM isolates in group A were completely identical. Comparison of the ITS sequence by Blast searching (http://www.ncbi.nlm.nih.gov) showed 100% similarity to a sequence from *Plectosporium* sp. RM1-12, and the

remaining highest matches were to sequences from other *Plectosporium* sp. The phylogenic relationships of the ITS region for the five NJM isolates and a total of 22 strains of related species of *Plectosporium* and *Verticillium* (including an outgroup strain) [23–26] were performed by NJ analysis (Fig. 9). The isolates of *Plectosporium* were divided into two major clades; one included the five NJM isolates (group A), *Plectosporium* sp. RM1-12, and *P. tabacinum* isolates, and the other included *P. alismatis* isolates and *P. delsorboi* isolates. In the former, the five NJM isolates, the three DAR isolates and the isolate RM1-12 formed a monophyletic clade, with a sister group comprising *P. tabacinum* supported by high bootstrap values, and this topology was similar to that of Wang et al. [24]. On the other hand, the latter were separated into *P. alismatis* isolates and *P. delsorboi* isolates, which was in accordance with the grouping of Zare et al. [25]. Therefore the five NJM isolates, three DAR isolates and RM1-12 were distinguishable from other species of *Plectosporium* on phylogeny.

The sequences of the ITS region and the β-tubulin gene of the two NJM isolates in group B were completely identical. The identity of the two isolates on the ITS region was compared by Blast searching which showed relative sequence homology to *E. maritima* CBS 491.71 (100%), and NBRC 9603 (100%), *E. minima* CBS 190.55 (99.1%), *E. pallida* CBS 624.73 (99.1%) and NBRC 9815 (99.1%), *E. stolkiae* CBS 159.71 (98.7%), *A. potronii* CBS 379.70F (100%) and *A. fuci* CBS 112868 (98.0%). Therefore the phylogenic relationships of the ITS region for them and a total of 22 strains of *Emericellopsis*, one of the teleomorphic genera found in terrestrial and marine inhabitants, *Acremonium potronii*, *A. fuci*, and *Bionectia* (including an outgroup strain) [27] were inferred from NJ analysis (Fig. 10). In the tree, the strains of *Emeriellopsis* and relative were divided into the four clades: clade A comprising *E. donezkii*, *E. humicola*, *E. microspora*, *E. minima*, *E. robusta*, *E. stolkiae*, and *A. tubakii*; clade B comprising *E. maritime*, *E. minima*, *E. pallid*, *E. stolkiae*, *A. fuci*, *A. potronii*, and the NJM isolates; and two minor clades C and D.

In the β-tubulin gene analysis, the two isolates had relative sequence homologies to *E. maritima* CBS 491.71 (97.2%), *E. minima* CBS 190.55 (98.2%), *E. stolkiae* CBS 159.71 (98.2%), *A. potronii* CBS 379.70 (97.2%), and *A. fuci* CBS 112868 (96.0%), and the NJ tree (Fig. 11) distributed the same topology as that from the ITS region analysis.

Based on the sequences of the ITS region and the β-tubulin gene, the two NJM isolates showed strong similarities to *E. maritima*, *E. minima* ss.str. (CBS

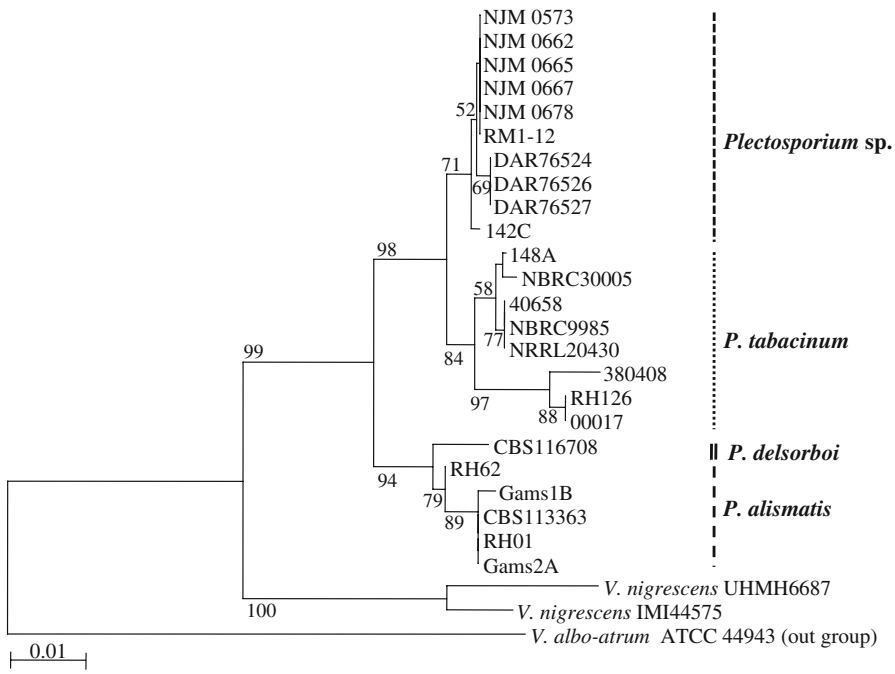


Fig. 9 Neighbor-joining tree based on sequences of the ITS region from 27 members of *Plectosporium* sp. and relatives; neighbor-joining algorithm with 1,000 bootstrap replicates (values >50 are shown with branches). *Verticillium albo-atrum* ATCC 44943 was taken as an outgroup

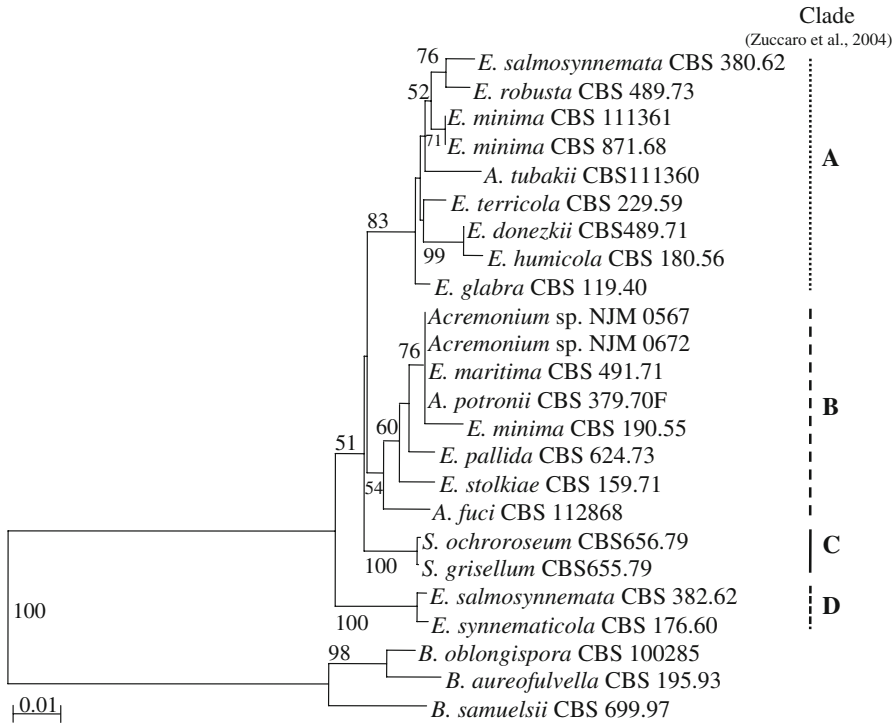


Fig. 10 Neighbor-joining tree based on sequences of the ITS region from 24 members of *Emericellopsis* sp. and relatives; neighbor-joining algorithm with 1,000 bootstrap replicates (values >50 are shown with branches). *Bionectira aureofulvella* CBS 195.93, *B. oblongispora* CBS 100285 and *B. samuelsii* CBS 699.97 were taken as an outgroup

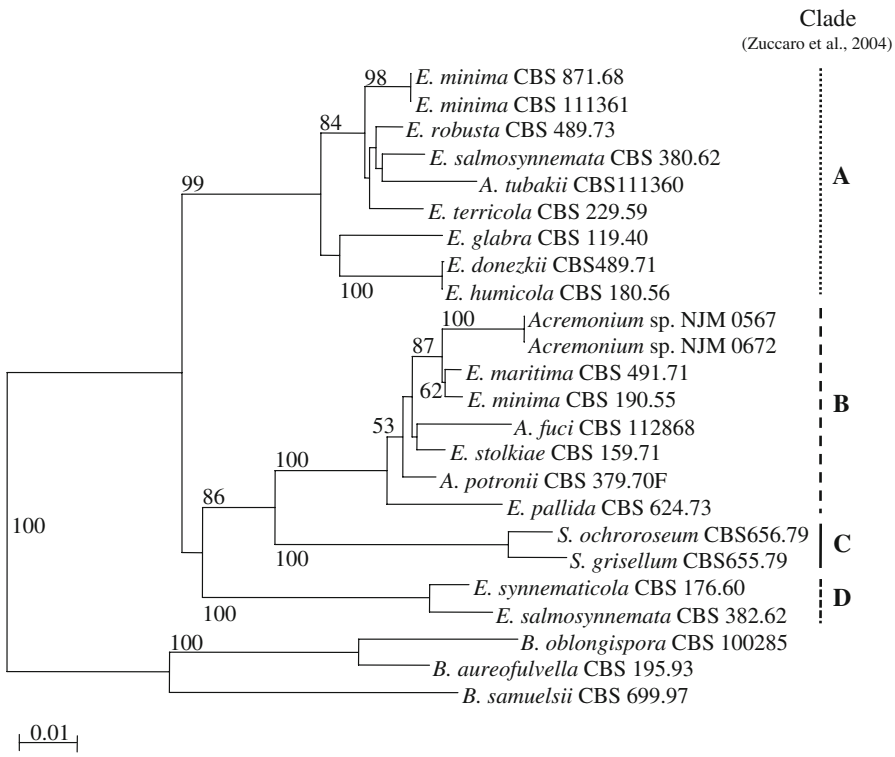


Fig. 11 Neighbor-joining tree based on sequences of the β-tubulin gene from 24 members of *Emericellopsis* sp. and relatives; neighbor-joining algorithm with 1,000 bootstrap replicates (values > 50 are shown with branches). *Bionectira aureofulvella* CBS 195.93, *B. oblongispora* CBS 100285 and *B. samuelsii* CBS 699.97 were taken as an outgroup

190.55, ex-type and unrelated to the other isolates), and *A. potronii*. However, the isolates did not produce ascospores on any of the tested media, so they were phylogenetically considered as *Acremonium* species with affinity to the *Emericellopsis* marine clade.

Sequence data for the seven NJM isolates and 2 NBRC strains of closely related species analyzed in this study were submitted to the DNA Data Bank of Japan (DDBJ) and the accession numbers were listed in Tables 1, 2. The alignment was deposited in TreeBASE (http://www.treebase.org/) with study accession number S2236.

Specific Identification of Isolates in Group A

Based on the phylogenetic analysis of ITS sequences, the five isolates of group A in this study were provisionally identified as a *Plectosporium* species. Upon further mycological examination on PYGS agar, the five isolates NJM 0573, 0662, 0665, 0667, and 0678 showed the same colony characteristics: initially dull white or slightly yellowish, moist, flat or slightly sulcate, becoming pale orange or grayish yellow in surface color and covered with trailing and interlacing hyphae with age. The isolates failed to grow at temperatures of 37°C and above. Examination of the slide culture preparations revealed conidiogenous cells that developed as discrete short phialides with a wavy tip. The conidiogenous cells also reduced to phialidic protrusions that developed as small pegs from the sides of hyphae. Conidia were one-celled, hyaline, ellipsoidal or cylindrical to obovoid, and smooth-walled.

A morphological comparison of isolate NJM 0662 with living cultures of two representative strains of *P. tabacinum*, NBRC 9985 (also as ATCC 24393, IMI 151458, CBS 367.73) and NBRC 30005, revealed that it belonged to a separate taxon, classifiable in the genus *Plectosporium* in Table 3, [28, 29]. Isolates NJM 0573, 0662, 0665, 0667, and 0678 were distinct from three known species: *P. tabacinum, P. alismatis* (Oudem.) W.M. Pitt et al., and *P. delsorboi* Antignani & W. Gams [26, 29]. We propose that the new taxon, *P. oratosquillae*, be established for these strains.

Plectosporium oratosquillae Pham Minh Duc, T. Yaguchi & Udagawa, sp. nov. (Figs. 12, 13, 14, 15, 16, 17, 18).

Etymology. The specific epithet refers to the host.

Coloniae in agaro "PYGS" lente crescentes, posto 14 dies 20°C, 10–13 mm diam attingentes, planae vel leviter sulcatae, ex mycelio vegetativo submerso constantes, funiculosae, dilute flavae ad dilute aurantiacae; conidiogenesis moderata ad abunda; reversum incoloratum vel dilute flavum. Mycelio ex hyphis hyalinis ad dilute flavo-brunneis, ramosis, levibus, septatis, 1–4 µm diam composito. Conidiophora ex hyphis submersis vel superficialibus oriunda, solitaria, simplicia vel parce ramosa, levia, hyalina. Cellulae conidiogenae phialidicae, hyalinae, rectae vel saepe sinuosae, cylindraceae vel subulatae, 6–18 × 2–4 µm, leves vel parum asperae, basaliter septatae, collari minuto cylindraceo usque ad 2 µm longo terminatae; tubi phialidici laterales, brevi-cylindracei, 3–4 × 1.5–2 µm, saepe basi septo nulli. Conidia unicellularia, hyalina, ellipsoidea vel cylindracea vel obovoidea, interdum curvata, 3–10 × 2–4 µm, levia, guttulata, in capitulis mucidis connexa. Chlamydosporae absentes.

Colonies on PYGS agar growing slowly, attaining a diameter of 10–13 mm in 14 days at 20°C, flat or slightly sulcate, consisting of a submerged vegetative mycelium, with surface developing of trailing and interlacing hyphae or ropes of hyphae, pale yellow (4A3) to pale orange (5A3); conidiogenesis moderate to abundant; exudate none; odor musty; reverse uncolored or pale yellow (4A3). Colonies on potato-carrot agar (PCA) growing more restrictedly, attaining a diameter of 15–16 mm in 21 days at 20°C, flat, thin, often centrally moist, with white cottony aerial mycelium in marginal areas, grayish yellow (4B4) to partially grayish orange (5B5); conidiogenesis light; reverse pale yellow (4A3). Colonies on Sabouraud glucose agar (SGA) growing more restrictedly, cottony, compact textured, rosy vinaceous; conidiogenesis sparse; reverse rosy buff.

Mycelium composed of hyaline to pale yellowish brown, branched, smooth-walled, septate, 1–4 µm diameter hyphae. Conidiophores arising from submerged or superficial hyphae, solitary, unbranched or sparingly branched, smooth-walled, hyaline. Conidiogenous cells phialidic, hyaline, straight or sinuous, cylindrical or subulate, 6–18 × 2–4 µm, smooth-walled or slightly roughened, basally septate, with a wavy tip, collarette cylindrical, up to 2-µm deep, sometimes inconspicuous; short phialidic protrusions arising laterally from intercalary cells of hyphae as small pegs, often lacking a basal septum, 3–4 × 1.5–2 µm. Conidia 1-celled, hyaline, pale yellowish brown in mass, ellipsoidal, cylindrical to obovoid,

Table 3 Morphological comparison of *P. oratosquillae* with known *Plectosporium* species

Feature	*P. oratosquillae*	*P. tabacinum*	*P. alismatis*[a]	*P. delsorboi*[a]
Colony diameter on PYGSA at 20°C, 14 d	10–13 mm	42–48 mm	ND	ND
Condiogenesis on PYGSA	Heavy	Light	ND	ND
Colony diameter on PDA at 25°C, 14 d	7–12 mm	58–65 mm	31–37 mm	63–91 μm
Conidiogenesis on PDA	Light	Heavy	Heavy	Heavy
Phialide length	6–18 μm	4–30 μm	12–35 μm	30–50 μm
Conidial shape	Ellipsoidal	Fusiform with rounded tapering ends	Fusiform with strongly tapering ends	Ellipsoidal
Conidial septation	0-Septate	0-1-Septate (1-septate often > 50%)	Mostly 1-septate	Mostly 1-septate
Conidial size	3−10 × 2−4 μm	8−12 × 2−3 μm (1-septate)	13−19.5 × 2.5−3.5 μm	6−13 × 3−3.5 μm
Chlamydospores	Absent	Absent	Present	Absent
Pathogenicity	Marine mantis shrimp	Plants of various kinds and cray fish (fresh-water fish)	Plants of Alismataceae	Plants of Zingiberaceae
Distribution	Japan	all over the world	Africa, Asia, Europe, North America, Oceania	Asia, Italy

[a] Data of *P. alismatis* and *P. delsorboi* from Pitt and Gams [28] and Antignani et al. [29], respectively

ND No data

sometimes curved, 3–10 × 2–4 μm, smooth-walled, guttulate, accumulated in a slimy head at the tips of phialides. Chlamydospores lacking.

Holotype: Dried colonies of IFM 56765 (NJM 0662), after 14 days growth on PYGS agar, deposited in the herbarium of Medical Mycology Research Center (MMRC), Chiba University, Chiba, Japan; isolated from diseased mantis shrimp (*Oratosquilla oratoria*), Yamaguchi Pref., Japan, April 2006.

Specific Identification of Isolates in Group B

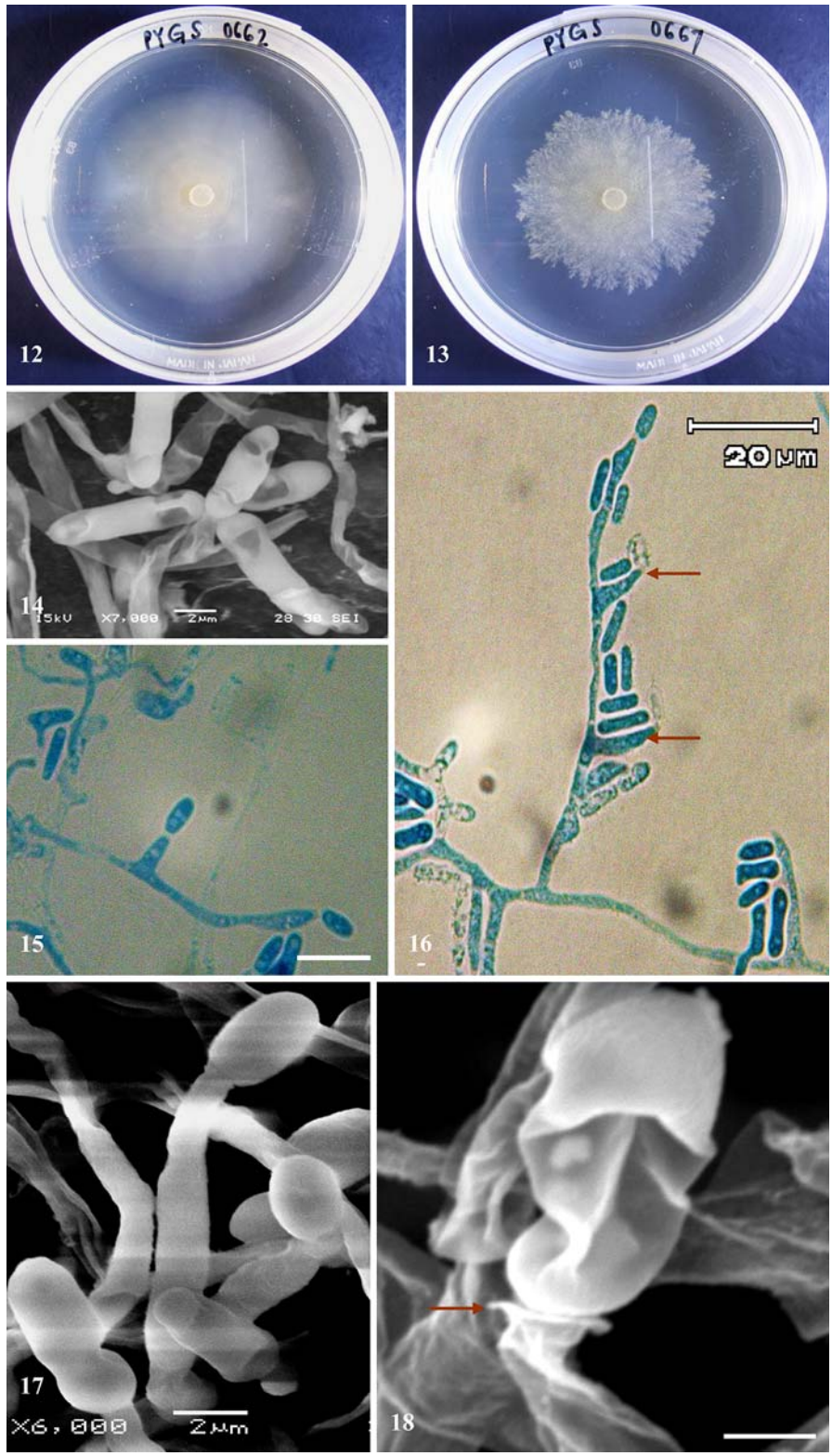
Although the failure of teleomorph development in culture was probably a result of unfavorable experimental conditions, phylogenetic analysis of ITS and β-tubulin sequences of the two isolates, NJM 0567 and NJM 0672, was identified to *Acremonium* sp. (the *Emericellopsis* marine clade). Their cultures on PYGS agar developed rapidly, with salmon-colored (Fig. 19) or pale orange (5A3) (Fig. 20), colonies attaining a diameter of 70 mm after 10 days at 20°C. Subcultures on PDA produced more or less floccose and rather smaller colonies, attaining a diameter of 20 mm in 7 days at 20°C. Conidia were one-celled, hyaline, cylindrical to ellipsoidal, straight or slightly curved, smooth-walled and measured 4–10 × 2.5–3 μm (Fig. 21), gathering in colorless slime at the tips of the phialides. Arising from the hyphae, conidiophores were macronematous and micronematous, simple or basitonously branched, septate, smooth-walled, 28–45 × 2–3 μm, and borne a terminal phialide (Figs. 22, 23). The mycelium was composed of hyaline, branched, septate, smooth, 1–4-μm-wide hyphae (Fig. 24). Phialide sometimes has septate (Fig. 25) and phialide apex with a visible collarette (Fig. 26).

Isolates were deposited as *Acremonium* sp. in the culture collection of the Medical Mycology Research Center, Chiba University, as IFM 56765.

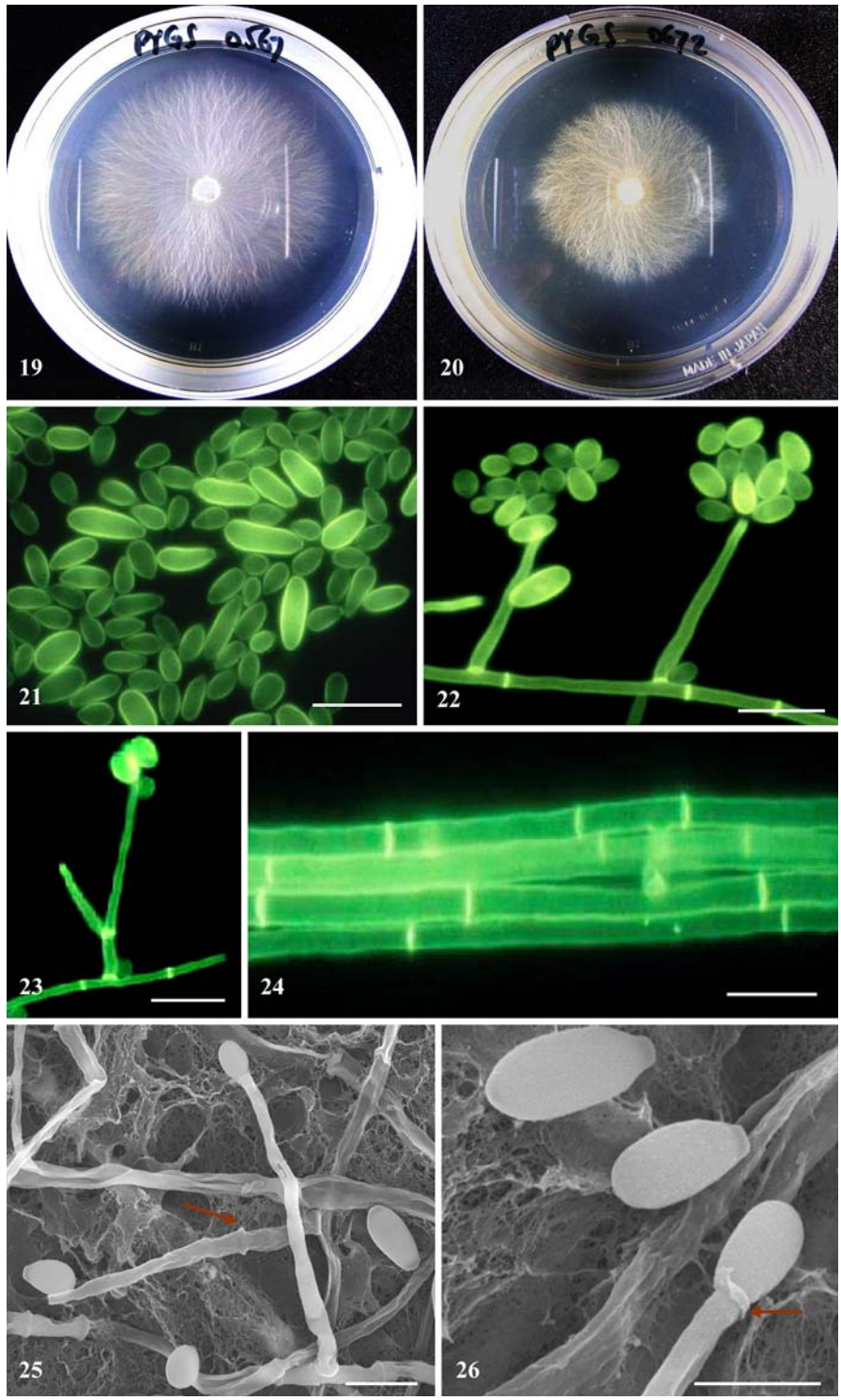
Physiology

Effect of Culture Media on Mycelial Growth of Isolates

The radial mycelial growth rates of isolates in *P. oratosquillae* and *Acremonium* sp. were evidently affected by culture media (Table 4). PYGS agar was apparently the most adequate for growth of all seven isolates tested. Besides the exceptional cases of more trace growth of the two isolates NJM 0662 and NJM 0678 on conventional media such as PDA, OA, and CMA, isolates in *P. oratosquillae* failed to grow on



Figs. 12–18 Morphological characteristics of isolates in the group A. **Fig. 12** Colony of NJM 0662 on PYGS agar at 25°C for 1 month; **Fig. 13** Colony of NJM 0667 on PYGS agar at 25°C for 25 days; **Fig. 14** Phialide apex aggregating elongate conidia, SEM; **Fig. 15** Short conidiophore, slide cultured, then stained with cotton blue, under a light microscope, bar = 10 μm; **Fig. 16** Elongate phialides and two short phialidic protrusions (arrows), slide cultured, then stained with cotton blue, under a light microscope; **Fig. 17** Elongate conidiophore, SEM; **Fig. 18** Phialide apex with collarette, SEM, bar = 2 μm



Figs. 19–26 Morphological characteristics of isolates in the group B. **Figs. 19** and **20** Colonies of NJM 0567 and 0672, both on PYGS agar at 25°C for 7 days; **Fig. 21** Obovoid to cylindrical conidia, slide cultured, then stained with B solution (fungal flora Y company, Japan), under a fluorescent microscope, bar = 10 μm; **Fig. 22** Erect conidiophores and conidia aggregating in a slimy head on phialide apex, slide cultured, then stained with B solution (fungal flora Y company, Japan), under a fluorescent microscope, bar = 10 μm; **Fig. 23** Branched conidiophore, slide cultured, then stained with B solution (fungal flora Y company, Japan), under a fluorescent microscope, bar = 10 μm; **Fig. 24** Hyphae aggregating in a strand with septa, slide cultured, then stained with B solution (fungal flora Y company, Japan), under a fluorescent microscope, bar = 8 μm; **Fig. 25** Phialides with septa (arrows), SEM, bar = 10 μm; **Fig. 26** Phialide apex with a visible collarette (arrow), SEM, bar = 5 μm

MEA, MSA, and SGA, which are usually adequate for most pathogenic and potentially pathogenic fungus cultures. This finding on the vegetative growth indicates that *P. oratosquillae* is an obligate marine fungus that can complete its life cycle only in marine environments. There is significant difference

Table 4 Effect of culture media on mycelial growth of isolates

Medium	Colony diameter (mm)						
	Plectosporium oratosquillae					*Acremonium* sp.	
	0573	0662	0665	0667	0678	0567	0672
PYGSA	17	16	9	16	9	60	49
PDA	–	1	–	–	1	27	28
MEA	–	–	–	–	–	24	21
OA	–	2	–	1	2	25	20
CMA	–	–	–	1	2	31	26
SGA	–	–	–	–	–	16	15
MSA	–	–	–	–	–	8	3

PYGSA Peptone yeast extract glucose seawater agar, *PDA* potato dextrose agar, *MEA* malt extract agar, *OA* oatmeal agar, *CMA* cormeal agar, *SGA* Sabroud glucose agar, *MSA* mycosel agar

Plectosporium oratosquillae: Colony diameter was measured at 25°C, 15 d after inoculation

Acremonium sp.: Colony diameter was measured at 25°C, 7 d after inoculation

–: No growth

in the growth response of *Acremonium* sp. isolates between PYGS agar and conventional media, and the salinity influence of seawater is obvious.

Effect of Temperature on Mycelial Growth of Isolates

The rates of radial mycelial growth of the seven isolates on PYGS agar are presented in Table 5. Most isolates in *P. oratosquillae* grow slowly at 10°C and rapidly at 20–25°C, but the decline in growth on each side of the optimum temperature is unequal. They have a very slow decline in their ability to growth at 10–15°C, whereas mycelial growth was hardly observed at 30°C. The two isolates of *Acremonium* sp. grow rapidly at 20–30°C, and the rates of radial mycelial growth of the seven isolates on PYGS agar were temperature dependent.

Effect of Concentrations of Artificial Seawater on Mycelial Growth of Isolates in P. oratosquillae

The rate of radial mycelial growth of all the five isolates belonging to *P. oratosquillae* followed similar trends in response to changes in seawater concentrations (Table 6). In comparison with maximum growth on 100% seawater agar, only slight decreases in colony diameter were observed at 75% seawater agar (its estimated salinity: 28.5‰). There is a general coincidence between salinity tolerance in culture of the isolates and their occurrence in sea environments: seawater from the Inland Sea, one of the sampling locations of diseased mantis shrimp, has a salinity range from 28.5 to 33.5‰.

Table 5 Effect of temperature on mycelial growth of isolates on PYGS agar

Temperature (°C)	Colony diameter (mm)						
	Plectosporium oratosquillae					*Acremonium* sp.	
	0573	0662	0665	0667	0678	0567	0672
5	1	2	–	–	–	12	1
10	5	8	3	3	1	24	10
15	11	13	5	13	4	37	17
20	17	15	7	16	6	52	31
25	18	16	9	18	8	66	48
30	3	1	5	–	2	40	34
35	–	–	–	–	–	4	16
40	–	–	–	–	–	–	–

Plectosporium oratosquillae: Colony diameter was measured at 25°C, 15 d after inoculation

Acremonium sp.: Colony diameter was measured at 25°C, 8 d after inoculation

–: <1 mm in diameter or no growth

Table 6 Effect of artificial seawater (ASW) concentration on mycelial growth of *Plectosporium oratosquillae* isolates

ASW	Colony diameter (mm)				
(%, v/v)	0573	0662	0665	0667	0678
100	17	16	9	16	10
75	17	14	6	14	9
50	9	11	5	9	7
25	6	7	3	2	4
12.5	5	2	2	1	2
0	–	1	–	–	–

Medium: Amended PYGS agar diluted by distilled water with a range from 75 to 0% ASW concentration; Salinity of 100% ASW was 38‰

Colony diameter was measured at 25°C, 15 d after inoculation

–: <1 mm in diameter or no growth

Effect of Initial pH on Mycelial Growth of Isolates

The effects of different pH levels on the vegetative growth of isolates in *P. oratosquillae* and *Acremonium*

Table 7 Effect of pH on mycelial growth of the isolates on PYGS broth

pH value	Relative mycelial growth						
	Plectosporium oratosquillae					*Acremonium* sp.	
	0573	0662	0665	0667	0678	0567	0672
3	0	0	0	0	0	0	0
4	0	0	1	0	1	1	1
5	1	1	1	1	1	2	3
6	2	2	2	2	2	3	3
7	3	3	3	3	3	3	3
8	3	3	3	3	3	3	3
9	3	3	3	3	3	3	3
10	3	3	3	3	3	2	3
11	1	2	1	0	1	1	3
12	0	0	0	0	0	0	0

Plectosporium oratosquillae: Values were determined by wet mycelial mass at 25°C, 10 d after inoculation

Acremonium sp.: Values were determined by wet mycelial mass at 25°C, 5 d after inoculation

Mycelial mass was shown as: 0 = no growth; 1 = weak growth; 2 = moderate growth; 3 = abundant growth

sp. are presented in Table 7. The minimum and maximum pH values for the growth of *P. oratosquillae* isolates were 5.0 (or 4.0 for the NJM 0665 and 0678) and 11.0 (or 10.0 for NJM 0667), respectively. The minimum and maximum pH values for the growth of *Acremonium* sp. isolates were 4.0 and 11.0, respectively. The pH of surface seawater at 25°C was about 8.0, which is within the optimum range 7.0–10.0 and 5–11 (or 6–9 for the NJM 0567) for the growth of *P. oratosquillae* isolates and *Acremonium* sp. isolates. A notable decline of growth occurred when pH decreased from 6.0 to 5.0. It is generally accepted that most fungi grow best in laboratory culture under slightly acid conditions, commonly pH 5–7, and so for most commercial media the pH is adjusted to 5.0–6.0. It is also well known that during growth some fungi may gradually alter the pH of the medium, with the extent and direction of this change depending on its constituents. However, in the comparative experiment between PYGS agar and conventional media (Table 4), other environmental factors, including initial pH in the culture media, may have countered the effects of seawater on the growth of *P. oratosquillae* isolates, but the effect is not severe.

Discussions

Histopathology

Based on histopathological observations, the fungi were regarded as pathogenic and the cause of disease in the Japanese mantis shrimp. In the agreement with the description of Bian and Egusa [30], melanized cuticular lesions appeared as granulomatous nodules, apparently due to the encapsulation of fungal hyphae by host haemocytes. In addition, the present result was in agreement with those studies on another anamorphic fungus infecting the gills of the kuruma prawn, *P. japonicus* [31], where fungal hyphae were also encapsulated and looked like granulomatous nodules. Conversely, the presence of fungal hyphae and conidia in tissue was not confirmed on the specimens of muscle or intestine.

Mycology

Phylogenetic Analysis

During a study on the molecular detection of ascomycetes associated with marine brown algae, Zuccaro et al. [27, 32] isolated unusual *Acremonium* species from healthy and decaying thalli of *Fucus serratus* in North Sea, Germany. To identify this *Acremonium* that might be closely related to *Emericellopsis*, phylogenetic analysis based on combined ITS and β-tubulin sequences was performed on 13 species of terrestrial and marine *Emericellopsis*, two of *Stanjemonium* (a terrestrial hyphomycete genus like *Acremonium*), and two marine-derived *Acremonium.* Their analysis showed that there were four monophyletic clades (A–D) in *Emericellopsis*, and allied anamorphic species. Of the two largest *Emericellopsis* clades, A and B, one was composed commonly of terrestrial isolates and the other of marine-derived isolates. The remaining two minor clades (C and D) all consisted of terrestrial isolates.

Specific Identification of Isolates in Group A

Plectosporium was introduced to accommodate *P. tabacinum* as the anamorph of *Plectosphaerella cucumerina* (Lindfors) W. Gams, which is a very common fungus, both in the rhizosphere and on decaying plant materials associated with soil. Palm

et al. [33], Domsch et al. [34] reviewed the distribution and pathogenicity of this fungus, which has been reported from a diverse range of hosts, including basil, chard, cucumber, melon, peanuts, potato, pumpkin, sugar beet, sunflower, tobacco, tomato, etc. Smith-Kopperl et al. [35] also reported that *P. tabacinum* was a pathogen of an invasive aquatic weed (*Hydrilla verticillata*) in Florida, USA. Surprisingly, it was isolated as a gill pathogen of crayfish (*Austropotamobius pallipes*), native to British freshwaters [10]. A second species, *P. alismatis* (Oudem.) W.M. Pitt, W. Gams & U. Braun with more falcate conidia, is commonly found on leaf spots of *Alisma* and other genera of the Alismataceae [26], and a third species, *P. delsorboi* Antignani et al. [29], with straight conidia, attacks the inflorescence of *Curcuma* (Zingiberaceae).

In Japan, *P. tabacinum* has been isolated from the aquatic sediment of a lake [36] and is more widely known as a blight pathogen of pumpkin leaves and stems, garden ranunculus seedlings, radish roots, etc. [37, 38]. However, to our knowledge, there is no previous report of the isolation of *Plectosporium* species from a marine environment.

Specific Identification of Isolates in Group B

In the monographs of the *Cephalosporium*-like hyphomycetes, by Gams [39, 40], *Acremonium* species were classified into three sections: sect. *Acremonium* with conidiophores consisting of unbranched phialides without a visible periclinal wall thickening and with collarette at the apex; sect. *Nectrioidea* with generally wider hyphae and often repeatedly branched conidiophores, the phialides often undulate in the upper part, with a progressive periclinal wall thickening and sometimes with a narrow collarette; and sect. *Gliomastix* (often treated as a separate genus) with usually pigmented conidia.

Acremonium has frequently been associated with the teleomorphic species of *Emercicellopsis* (Bionectriaceae, Hypocreales) and several nectriaceous genera. The *Emercicellopsis* species appear to be primarily soil saprophytes that are characterized by non-ostiolate globose ascomata with transparent peridia but appearing dark because of the pigmented ascospores within eight-spored and globose asci, and one-celled, ovoid to ellipsoidal, dark-walled, wing-like ornamented ascospores. They can be isolated from various environments worldwide, including cultivated and forest soils, rhizospheres, mycorrhizae, and freshwater-, estuarine- and marine sediments [33]. During a survey of fungi associated with mud in marine, brackish lake and freshwater habitats in Japan, Tubaki [41] noted that *E. humicola*, *E. microspora*, and *E. minima* were isolated frequently, and suggested that aquatic sediments might be characteristic environments for some members of this genus.

In the *Acremonium* anamorphs of *Emericellopsis*, rather complex conidiophores occur that are unlike other species of *Acremonium.* The branched conidiophores suggest *Acremonium* sect. *Nectriodea,* but hyphae and conidiophores are thin-walled. The morphological features of the two isolates in Group B have a branching phialide (conidiophore) structure and relatively large ellipsoidal conidia that are nearly identical to those of the *Emericellopsis* anamorphs. Furthermore, with the exception of ascomatal production, sizes of the phialides and conidia of the isolates suggest a possible relationship to *E. minima ss.str.*; viz., the phialides of *E. minima* are 20–30 × 2.0–2.5 μm and the conidia are 4–10 × 2.0–3.5 μm (fide Stolk, [42]), whereas in the isolates, phialides are 28–45 × 2.0–3.0 μm and conidia are 4–10 × 2.5–3.0 μm.

In contrast, two marine-derived *Acremonium*, *A. potronii and A. fuci*, are distinguished by differences in phialide and conidium morphology. In *A. potronii*, phialides are 11–27 × 1.0–2.0 μm, and conidia are obovoid, 2.1–4.0(–5.0) × 1.3–2.5 (−3.0) μm [39, 40]. In *A. fuci*, phialides are 7.5–23.5 × 1.0–2.2 μm, and conidia are obovoid to broadly ellipsoidal, 10–15 × 3.0–6.0 μm [32].

Physiology

Effect of Culture Media on Mycelial Growth of Isolates

These results might be important when considering the ecological distribution of *P. oratosquillae* and *Acremonium* sp. (the *Emericellopsis* marine clade). According to Jones and Jennings [43] in their comparative study on vegetative growth of ten marine, one freshwater and two terrestrial species in media made up with distilled water and seawater, indicated that the response of the marine fungi varied

among species, and in only one instance was the dry weight of vegetative mycelium significantly greater in a distilled water medium. The non-marine fungi all produced significantly greater dry weights of vegetative mycelium in the distilled water medium. However, Jones and Jennings stated that a simple comparison of growth in distilled water with seawater media did not give a complete picture of the growth of fungi under saline conditions. They further suggested that the close association of marine fungi with the sea cannot be ascribed alone to the influence of seawater on vegetative growth. Jones studies some of the factors influencing biodiversity of marine fungi [44]. He found that salinity was important in affecting species composition and that marine fungi could have either a wide salinity tolerance (such as *Asteromyces cruciatus*, salinity 0–100% sea water) or a narrow salinity tolerance (such as *Althrornia crouchi*, salinity 40–100% sea water).

On the other hand, there is good evidence that certain species may require seawater for fruiting. The effects of salinity on sporulation and spore germination of a range of terrestrial, freshwater, and marine fungi were investigated by Byrne and Jones [45, 46]. Their results showed that reproduction and spore germination of the marine fungi tested were markedly affected by high salinities. A similar trend was found in our description for *P. oratosquillae*, which only produced abundant conidia in the presence of seawater, which supports the point that the fungus is obligatorily marine.

Effect of Temperature on Mycelial Growth of Isolates

Boyd and Kohlmeyer [47] reported the growth of three marine hyphomycetes on PYGS medium: *Asteromyves cruciatus* Moreau & Moreau ex Hennebert, *Sigmoidea marina* Haythorn & Jones, and *Varicosporina ramulosa* Meyers & Kohlm. Their study demonstrated that growth tests of temperature tolerance could be used to indicate possible geographic distribution patterns of marine fungi. For example, *V. ramulosa* is a tropical–subtropical species and its maximum growth occurs between 35 and 40°C, whereas *A. cruciatus* and *S. marina* are temperate water species; the former species is observed in the North Atlantic and North and South Pacific and grows at 10°C, but with optimum growth at 20°C and no growth at 35°C.

The data on temperature requirements for optimum growth of Group A isolates showed that *P. oratosquillae* is evidently a member of a temperate water species. In fact, the occurrence of diseased mantis shrimp was found to be in the temperate seawater zone of the Pacific side of central Japan, where the temperatures of bottom seawater in the crustacean habitats (at Tokyo Bay) ranged from 12.7°C (the lowest in March) to 22.3°C (the highest in September) [48]. Although the range of Group B isolates extends further toward the subtropical zones than that of *P. oratosquillae*, our results suggest that *Acremonium* sp. (the *Emericellopsis* marine clade) is also a temperate water species.

Effect of Concentrations of Artificial Seawater on Mycelial Growth of Isolates in P. oratosquillae

Marine fungi are considered to be species that are capable of indefinitely repeating their lifecycle in the fully saline conditions of the sea, but which may also continue to develop in other conditions, including those of fluctuating salinity in estuaries. Tubaki and Ito [49], surveyed fungi grown on submerged balsa wood and bamboo blocks in brackish estuarine habitats (salinities of their locations: 1.6–11.0‰) in Japan. They encountered 24 species of ascomycetous and anamorphic fungi and examined the mycelial growth of 17 brackish water-inhabiting species at different concentrations of natural seawater in laboratory culture. They found that most of the brackish water-inhabiting fungi exhibited a broad tolerance to decreasing salinities, with vegetative growth and reproduction the most markedly affected; 10 species responded favorably to salinities above 10% seawater, with maximum growth generally at 30–50% seawater, and three species were characteristic in approximately equal growth from 30 to 100% seawater. Moreover, in the case of three true marine fungi, such as *Corollospora maritima* (Linder) Kohlm., *Leptosphaeria oraemaris* Linder, and *Torpedospora radiata* Meyers, maximum growth occurred in 70 to 100% seawater. Thus, the effects of salinities above 70% seawater on the maximum growth of marine fungi could account for their distribution in the higher salinity regions of an estuary or in the sea. The vegetative growth of *P. oratosquillae* exhibited a similar response to that of these three marine fungi, because the colony development of isolates in Group A was severely

inhibited at low salinity (12.5–25% seawater agar) whereas they exhibited optimum mycelial growth at 75–100% seawater (almost normal seawater). This may be important when considering the epidemic distribution of the mantis shrimp pathogen.

Effect of Initial pH on Mycelial Growth of Isolates

The two isolates of *Acremonium* showed similar trends in their response to pH changes as *P. oratosquillae* (Table 7), but their visible growth was observed over a wide pH range. They were also more tolerant to high temperature (Table 5), suggesting that this fungus is cosmopolitan, not only as a pathogen of mantis shrimp but also as a saprophyte in aquatic and terrestrial environments.

It is difficult to adequately state the general influence of pH on fungal ecology, particularly as its effects are modified by so many other environmental factors. For example, although there is some evidence that alkaline and acidic soils contain different fungal communities, this does not always correlate with the pH-growth responses of representative species of these communities in laboratory experiments. Reports on the distribution of *P. tabacinum* were related almost exclusively to cultivated land [33]. By means of distribution studies using alkaline and slightly acidic media [i.e., alkaline CMA (pH 9.7) and CMA (pH 6.0)], Nagai et al. [50] noted that *P. tabacinum* was isolated only on alkaline CMA from weakly acidic forest soil (pH 5.0–6.0) in Japan. Even though most plant pathogens grow best in media with an initial pH of 5.0–6.5, and mycelial growth of *P. tabacinum* occurred over a range of pH 4–8 [51], Nagai et al. confirmed that optimum growth of the Japanese isolate of *P. tabacinum* occurred at pH 7.0 on MEA. They also showed that many *Acremonium* species were isolated from Indonesian alkaline soil, and the growth patterns of these alkalophilic isolates were the same as that of the *Acremonium* isolate from Japanese acidic soil.

Conclusion

The Japanese mantis shrimp (*O. oratoria*) lives on the mud of the lower intertidal zone in coastal waters around Japan. The crustacean is usually found in U-shaped burrows in soft mud, swimming along the surface of the intercoastal waterway at night.

Since the 1980s, significant case reports on the occurrence of black gill disease and disease-associated mortality of the mantis shrimp have increased throughout the Pacific coast side of Japan. Even though there has been considerable concern over the disease, the cause has yet to be unequivocally identified. Thus, two hyphomycetes have been isolated from the diseased samples, collected from Yamaguchi and Aichi Prefectures on the Pacific coast side of Japan and are taxonomically described as etiological agents: *P. oratosquillae* and *Acremonium* sp. (a member of the *Emericellopsis* marine clade). *P. oratosquillae*, a new obligate marine fungus, is characterized by very slow growth on PYGS agar with optimum growth at 20–25°C, while the culture of *Acremonium* sp. displays some different responses to temperature (optimum: 20–30°C), salinity tolerance, and acidity (optimum pH: 5–10). There are no substantial ecological data on *P. oratosquillae* in the coastal zone [52], but numerous fungi, including *Emericellopsis* species, are more common in the coastal sediments of high organic matter than in those of lower organic content [36, 43].

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