

Phylogenetic position of freshwater and marine *Sigmoidea* species: introducing a marine hyphomycete *Halosigmoidea* gen. nov. (Halosphaeriales)

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Abstract

The aquatic hyphomycete genus *Sigmoidea*, with freshwater and marine representatives, is re-examined at the morphological and molecular levels. Currently six species are accepted, four from freshwater habitats (*S. prolifera*, *S. aurantiaca*, *S. contorta*, *S. praelonga*) and two marine species (*S. marina*, *S. luteola*). Phylogenetic analyses of the ribosomal small subunit rRNA gene sequences of freshwater and marine *Sigmoidea* species indicate that the marine species are distantly placed from the freshwater species, *S. prolifera* and *Pseudosigmoidea cranei*. The latter species are placed in the Dothideomycetes, in sister clades, with 81% bootstrap support. The phylogenetic placement of both marine *Sigmoidea* species, inferred from large subunit rRNA gene sequences, was within the genus *Corollospora* (Halosphaeriales, Sordariomycetes, Pezizomycotina). An undescribed *Sigmoidea* species isolated from the Baltic Sea and one previously incorrectly identified as *S. marina* from Japan (*S. parvula* sp. nov.) formed a clade together with *Corollospora quinquesepata* and *Varicosporina ramulosa*, and were located in a sister group to *S. luteola* and *S. marina*. Based on morphological and phylogenetic evidence, *Halosigmoidea* gen. nov. is proposed to accommodate the

three marine *Sigmoidea* species (*S. luteola*, *S. marina*, *S. parvula* sp. nov.). Identification keys to the genera *Halosigmoidea*, *Pseudosigmoidea* and *Sigmoidea*, and *Halosigmoidea* species are provided.

Keywords: 18S; 28S; *Corollospora*; *Halosigmoidea* gen. nov.; Halosphaeriales; ITS rDNA; marine fungi; *Sigmoidea*.

Introduction

Crane (1968) established the genus *Sigmoidea* for a submerged freshwater hyphomycete with hyaline, scolecoform conidia, *Sigmoidea prolifera* (R.H. Petersen) J.L. Crane that was originally placed in the genus *Flagellospora* Ingold by Petersen (1963). The establishment of the genus *Sigmoidea* was based on the presence of holoblastic rather than phialidic conidiogenous cells (Crane 1968, Marvanová and Descals 1987). Since then, three additional freshwater species have been described: *S. aurantiaca* Descals, a taxon having wide falcate to sigmoid shaped conidia (Descals and Webster 1982); *S. praelonga* Marvanová, from Czech waters, with longer conidia than *S. prolifera* (Marvanová 1986) and *S. contorta* Marvanová et Hywel-Jones, a pleoanamorphic species with macroconidia that have sympodial or rarely percurrent conidiogenous cells and polyblastic microconidia borne on contorted thick-walled hyphal branches (Marvanová and Hywel-Jones 2000).

Crane (1968) erected the genus *Sigmoidea* using Petersen's holotype specimen preserved in NY as type. However, Crane's specimen, 85-B-65 (=ATCC 16660), also deposited as *S. prolifera*, differed from the type in not showing ampulliform sympodially elongating conidiogenous cells (Marvanová and Descals 1987). In 2000, Ando and Nakamura transferred Crane's specimen to a new genus, *Pseudosigmoidea* K. Ando et N. Nakamura with *P. cranei* K. Ando et N. Nakamura, as the type species (ATCC 16660), based on the presence of polyphialdic enteroblastic conidiogenesis. Although there is no doubt that these two specimens represent two different taxa, there is no evidence of polyphialdic enteroblastic conidiogenesis from the illustrations in the protolog by Ando and Nakamura, and there is no indication of repeated production of conidia from the same conidiogenous locus. The taxonomic position of these specimens therefore remains unclear.

Two additional *Sigmoidea* species with sympodial conidiogenesis cells, curved (not sigmoid as indicated in the original descriptions) conidia with the end cells lacking cytoplasmic contents were described: *Sigmoidea marina* Haythorn et E.B.G. Jones (Haythorn et al. 1980)

and *Sigmoidea luteola* Nakagiri et Tubaki (Nakagiri and Tubaki 1982). The teleomorph of *S. luteola* is *Corollospora luteola* Nakagiri et Tubaki (Nakagiri and Tubaki 1982), while no teleomorph is known for *S. marina*. An additional *Sigmoidea* species, *S. littoralis* Phillips, collected from decomposing material of the red alga *Gelidium coulteri* Harv., from Hopkins Marine Station, Pacific Grove, California in 1979, was listed in *Index fungorum* (CABI website, this reference has now been removed), but to our knowledge a description of this species has never been published nor has an isolate been deposited anywhere. Additional collections were made from *Rhodoglossum affine* (Harv.) Kyl. in California and *Prasiola meridionalis* S. et G. at Cattle Point, San Juan Island, Washington (G.K. Phillips, personal communication); unfortunately, no specimens or isolates are available for morphological or molecular analysis.

During a study of fungi associated with marine plants and algae in Germany, many isolates belonging to the genus *Sigmoidea* were recovered from *Fucus serratus* L. collected on Helgoland, North Sea (Zuccaro et al. 2003, 2008) and identified as *Sigmoidea marina*. Others collected from a variety of substrata located at different sites in the Baltic Sea also could not be identified according to the descriptions of known species (Crane 1968, Haythorn et al. 1980, Descals and Webster 1982, Nakagiri and Tubaki 1982, Marvanová 1986, Marvanová and Descals 1987, Marvanová and Hywel-Jones 2000). These isolates, together with three *Sigmoidea* sp. (originally identified as *S. marina*) collected from sea foam in Japan, shared several morphological characteristics typical of *S. marina*, but differed by having shorter conidia (Nakagiri 1989). Because the marine *Sigmoidea* species are morphologically different from *S. prolifera* in having predominantly C- or U-shaped conidia with end cells lacking cytoplasm, irregular sympodial conidiogenesis cells, and are marine in habitat, a molecular phylogenetic study was undertaken to resolve the taxonomic relationship between the freshwater and marine species.

Materials and methods

Fungal cultures

Fungi used in this study are *Corollospora luteola* NBRC 31315 (ex-type) isolated from ascospores in sea foam (Nakagiri 1989), *Pseudosigmoidea cranei* ATCC 16660 (ex-type), *Sigmoidea prolifera* CCM F-10002 (isolated and kindly donated by Dr. Ludmilla Marvanová), *Sigmoidea marina* NBRC 103271 (=AN-690, G. Jones, PP 0423 ex-type), and *Sigmoidea* sp. Japan (*Halosigmoidea parvula*) NBRC 32159, NBRC 32160, and NBRC 103558 (ex A. Nakagiri). Isolates TUB 6582, TUB 6989, and TUB 407 of the undescribed *Sigmoidea* sp. (*H. parvula*) were collected, respectively, from Ahrenshoop, Wismar, and Travemünde sites in the Baltic Sea in September 2002, March 2003, and May 2004. Two of the isolates (TUB 6582 and TUB 407) were recovered from red algae (*Polysiphonia* sp. and *Ceramium* sp., respectively) and one (TUB 6989) from the seagrass *Zostera marina* L. All cultures recovered from algae were isolated after surface sterilization of host material (method described in Zuc-

caro et al. 2003). Ten strains of *S. marina* (*Halosigmoidea marina*), were isolated from *Fucus serratus* thalli collected during a study on Helgoland, North Sea, of which TUB 270 was used in this study. TUB 156, isolated from Norddeich, North Sea was also used in this study. Two *Sigmoidea* sp. isolates (TUB 7952 and TUB 7956) were isolated from *Enteromorpha* sp. from Niendorf, Baltic Sea. Four isolates of *Corollospora angusta* Nakagiri et Tokura were isolated from *F. serratus* collected from Helgoland, North Sea, but only TUB 1 was used in this study. Other freshwater *Sigmoidea* species were not available to us for study as existing isolates were subject to commercial confidentiality.

Fungi were maintained on freshwater or seawater cornmeal agar (Difco, Criterion, Santa Maria, USA: 30 g l⁻¹ maize meal, 20 g l⁻¹ agar) as appropriate. Cultures were grown in GYP broth (homemade: 4 g l⁻¹ glucose, 4 g l⁻¹ yeast extract, 2 g l⁻¹ peptone) in filtered natural seawater except for *Sigmoidea prolifera* and *Pseudosigmoidea cranei*, which were grown in a CMA freshwater medium. Fungal biomass was harvested by filtration and washed with sterile distilled water.

Isolates TUB 6989 and TUB 407 were cultured on artificial seawater (Meersalz, Wiegandt GmbH, Krefeld, Germany) glucose-peptone-yeast extract agar GYP (0.1 g l⁻¹ glucose, 0.1 g l⁻¹ yeast extract, 0.5 g l⁻¹ peptone, 20 g l⁻¹ agar, 15 g l⁻¹ sea salt) at 14, 20, and 25°C in the dark for morphological description. For molecular analysis, these two isolates were grown on glucose-peptone-yeast extract agar (0.1 g l⁻¹ glucose, 0.1 g l⁻¹ yeast extract, 0.5 g l⁻¹ peptone, 20 g l⁻¹ agar, 15 g l⁻¹ sea salt) at room temperature for 1 week.

DNA isolation and PCR amplification

For the strains isolated from Germany, up to 500 mg of fresh mycelium was removed from the surface of the agar plates with a sterile loop and extracted using the FastDNA SPIN Kit for soil (BIO 101 Systems, Macherey-Nagel, Düren, Germany). DNA fragments were used as templates for the polymerase chain reaction (PCR) using the 28S rDNA primers NL209–NL912 (Zuccaro et al. 2003) or the ITS primers ITS1–ITS4 (White et al. 1990). PCR amplifications were performed on a Biometra T personal PCR system (Göttingen, Germany) as described by Zuccaro et al. (2003). The PCR samples were purified using a GENECLEAN III Kit (BIO 101 Systems) and run in an agarose gel together with a custom-made standard to check the DNA concentration. Cleaned PCR products were sequenced using the fluorescent method in a LICOR 4200 DNA sequencer (Lincoln, NE, USA).

The cetyl-trimethyl-ammonium-bromide (CTAB) method for genomic DNA extraction (Jones et al. 2006) was used to obtain DNA from the remaining strains.

The nuclear small subunit rRNA gene was amplified from genomic DNA using the primers NS1/NS6 (or /NS4) and NS5/NS8 (or /ITS4) (White et al. 1990), whereas the large subunit rRNA gene was amplified using primers JS1/JS8 (or /LR7) (Bunyard et al. 1994, Landvik 1996). Both SSU and LSU rDNA were amplified using a FINN-ZYMES, DyNAzyme™ II DNA Polymerase Kit (Macherey-Nagel, product code F-551S) in a Perkin Elmer thermal cycler (P.E. Applied Biosystems, USA). The amplification

cycle consisted of an initial denaturation step at 94°C for 5 min followed by 35 cycles of (i) denaturation (94°C for 1 min) (ii) annealing (55°C for 1.5 min), and (iii) elongation (72°C for 2.5 min), and a final 10 min elongation step at 72°C. The PCR products were analyzed by agarose gel electrophoresis and purified using a NucleoSpin® Plant DNA Purification Kit (Macherey-Nagel, Catalogue No. 740 570. 50).

DNA sequencing

Direct sequencing of purified PCR products was performed using NS1, NS2, NS3, NS4, NS5, and NS8 of SSU rDNA (White et al. 1990), while JS1, JS5, JS8, and LR7 were used to sequence the LSU rDNA (Landvik 1996) with an ABI PRISM dRhodamine Terminator Cycle Sequencing Kit (Macrogen Inc., Korea). Reactions and programs were chosen according to the manufacturer's recommendations. Samples were analyzed automatically by the BIOTEC Service Unit (BSU, Pathumthani, Thailand) laboratory or using the fluorescent method in a Li-COR 4200 DNA sequencer.

Sequence alignment and phylogenetic analysis

After sequence assembly, sequences were aligned manually in Se-Al v1.0a1 with other sequences obtained from GenBank (Rambaut 1999). Three datasets, SSU, LSU, and ITS-5.8S rDNA were analyzed in PAUP 4.0b10 using maximum parsimony and maximum likelihood criteria (Swofford 2002).

For the SSU dataset, 59 taxa were included. A unique insertion of 282 bases in *Pseudosigmoidea cranei* ATCC 16660 was excluded from analysis (Figure 1). *Candida atlantica* (Siepmann) S.A. Mey et Simone and *Kluyveromyces aestuarii* (Fell) van der Walt were designated as the outgroup taxa. The best tree(s) under parsimony criterion was (were) found through a heuristic search [starting tree via stepwise addition, 100 replicates of random sequence addition, tree-bisection-reconnection (TBR) branch-swapping algorithm]. Support of the clades was estimated by 1000 parsimony bootstrapping through a heuristic search (starting tree via stepwise addition, 10 replicates of random sequence addition, TBR).

For the LSU dataset, 18 taxa were included, with *Petriella setifera* (J.C. Schmidt) Curzi and *Microascus trigonosporus* C.W. Emmons et B.O. Dodge as the outgroup taxa. The best tree(s) under parsimony criterion was (were) found through a heuristic search (starting tree via stepwise addition, 100 replicates of random sequence addition, TBR branch-swapping algorithm) (Figure 2). Support of the clades was estimated by 1000 parsimony bootstrapping through a heuristic search (starting tree via stepwise addition, 10 replicates of random sequence addition, TBR).

The ITS-5.8S rDNA dataset comprised 12 taxa belonging to the genera *Sigmoidea* and *Corollospora* for 743 nucleotides covering both ITS regions and the 5.8S rRNA gene, and two sequences from *Ceriosporopsis halima* Linder as the outgroup taxon. Maximum likelihood and parsimony analyses were performed using this dataset. In both analyses, absent sequence information was treated as missing. For the maximum likelihood analysis, a

user-specified substitution matrix, calculated from the data matrix, and an estimated shape parameter were used, consistent with a GRT+G model. The maximum parsimony analysis was conducted using a heuristic search with random stepwise sequence addition (10 replicates) and TBR branch-swapping. Support for clades was estimated by bootstrapping (100 replicates) through heuristic searches (starting tree via stepwise addition, 10 replicates of random sequence addition).

Results

Phylogenetic analysis

The SSU dataset comprised 2122 characters with 455 parsimony-informative characters. In order to determine the ordinal affinity of the freshwater and marine *Sigmoidea* species, sequences from representative orders of the Dothideomycetes and Sordariomycetes were analyzed with those of *Pseudosigmoidea cranei*, *Sigmoidea prolifera*, *Corollospora luteola*, *Sigmoidea marina* (*Halosigmoidea marina*), and *Sigmoidea* sp. (*Halosigmoidea parvula*). Two most parsimonious trees (MPTs) resulted (tree length=1759, C.I.=0.5088, R.I.=0.7078) and the consensus tree is shown in Figure 1.

Both *Pseudosigmoidea cranei* and *Sigmoidea prolifera* are positioned in the Dothideomycetes. These two species occur in a fairly well supported clade (81%) with the hyphomycetes *Helicoon fuscosporum* Linder, *Troposporella fumosa* P. Karst., and *Phaeotrichum benjaminii* Malloch et Cain (Phaeotrichaceae). The relationship between these taxa and other orders of the Dothideomycetes is unclear, as indicated by the low bootstrap values on the internal branches.

Monophyly of the Sordariomycete taxa is well supported (100%) and the marine *Sigmoidea* species are distantly related to the freshwater *Sigmoidea* species and reside in the order Halosphaeriales (Figure 1). *Corollospora luteola*, *Sigmoidea* sp. Japan (*Halosigmoidea parvula*) and *Sigmoidea marina* (*Halosigmoidea marina*) are closely related to the genus *Corollospora*.

Analysis of the nuclear partial LSU rRNA was used to resolve the phylogenetic affinity of *Halosigmoidea* spp. (*Corollospora/Sigmoidea luteola*, *Sigmoidea marina*, *Sigmoidea* sp. Japan, *Sigmoidea parvula*) with *Corollospora* species. The consensus tree of two MPTs is shown in Figure 2 (tree length=948, C.I.=0.6108, R.I.=0.6766). This is a strict parsimony consensus tree with 18 isolates and with *Petriella setifera* and *Microascus trigonosporus* as an outgroup. All the marine *Sigmoidea* isolates group within the *Corollospora* clade with good bootstrap support and fall into two distinct subclades: (1) *S. marina* is monophyletic with two isolates from Germany and one from the UK (ex-type), with *Corollospora angusta* Nakagiri et Tokura as a sister group (clade A) with *C. luteola* basal to clade A. (2) *Halosigmoidea parvula* strains (clade B) are monophyletic with *Corollospora quinquesepata* Nakagiri and *Varicosporina ramulosa* Meyers et Kohlm. as a sister group, but with weak support (50%). Two *Corollospora maritima* Werderm. strains form a monophyletic group (clade C), while clade D contains a range

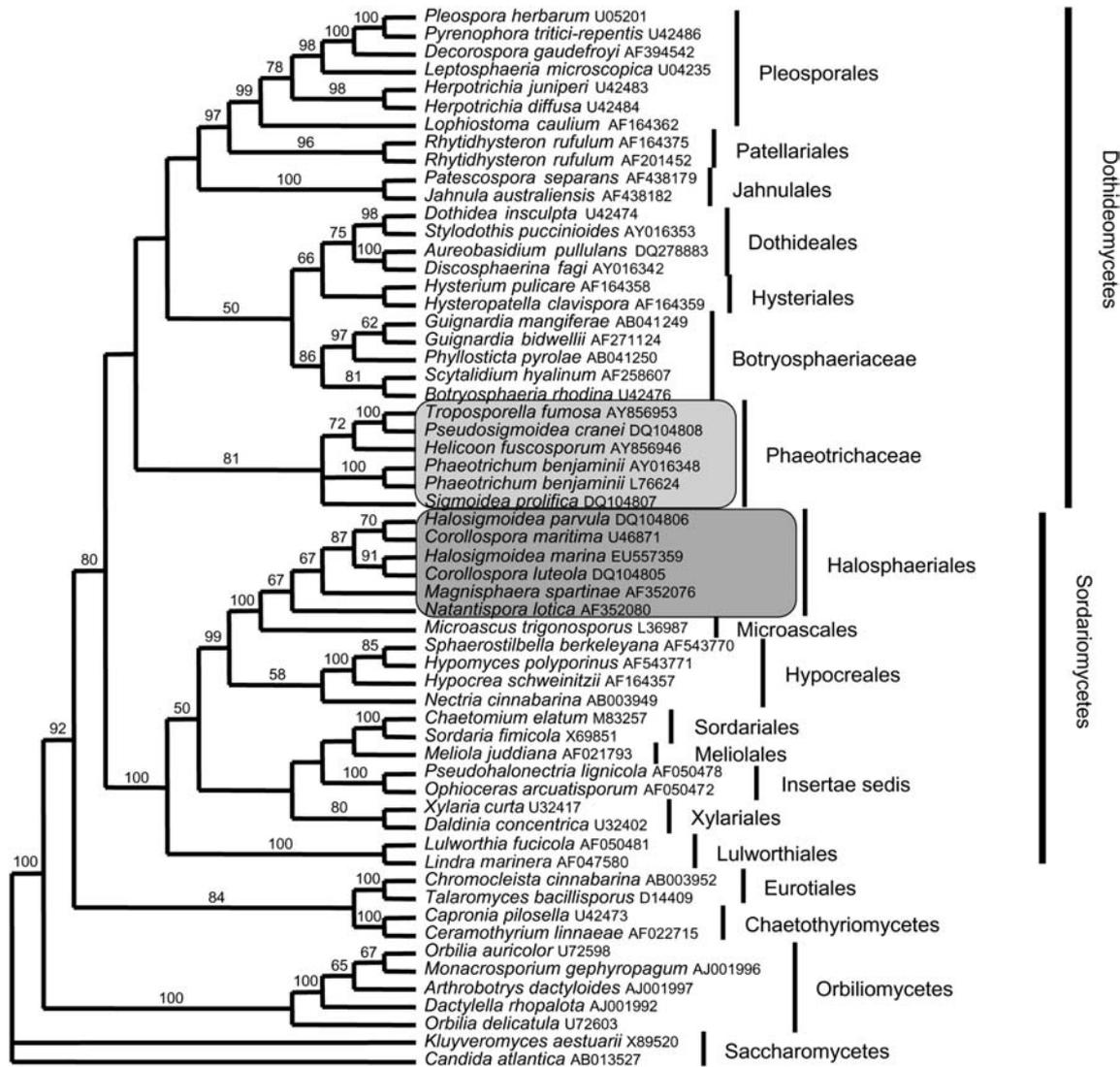


Figure 1 One of the consensus parsimonious trees inferred from SSU rDNA sequences. Maximum parsimony bootstrap values $\geq 50\%$ are indicated above the branches.

of *Corollospora* species, all with phragmoseptate ascospores.

The ITS-5.8S rDNA analysis revealed the cohesiveness of the *Halosigmoidea parvula* clade compared to clades containing other *Halosigmoidea* and *Corollospora* species (Figure 3). The dataset comprised 743 characters with 288 parsimony-informative sites, and it generated two most parsimonious trees of length 547 with C.I.=0.927, R.I.=0.934, R.C.=0.866, and H.I.=0.073, with *Ceriosporopsis halima* strains as the outgroup. Maximum likelihood analysis, using the GTR+G model with estimated nucleotide frequencies and substitution rates, generated a tree with a topology similar to that obtained using parsimony criterion. Both analyses revealed three strongly supported clades: (1) *H. parvula* isolates from Germany and Japan, (2) *Halosigmoidea luteola*, *Halosigmoidea marina*, *Corollospora intermedia* I. Schmidt and *Corollospora angusta*, and (3) two undescribed *Halosigmoidea* strains with *Corollospora colossa* Nakagiri et Tokura. The proportion of site variation between selected *Halosigmoidea* species is shown in Table 1. Little variation exists between *H. parvula* isolates from Germany

and Japan, indicating an extremely close evolutionary relationship, whereas it ranges from 0.29 to 0.34 between other species.

Discussion

A number of studies deal with the taxonomy, ultra-structure, and phylogeny of *Corollospora*, a genus with a large number of species in the Halosphaerales (currently 19, while others await description; E.B.G. Jones, personal observations) (Jones et al. 1983, 2009, Campbell et al. 2002, Hsieh et al. 2007, Abdel-Wahab et al. 2009). However, no clear picture emerges as to the phylogenetic relationships among the various species. Certainly the molecular data suggest that, while *Corollospora* species form a well-supported monophyletic group, there is great sequence variation within the clade and this may well represent distinct lineages. Morphologically, there is great variation between different taxa in the genus (Jones 1995, Campbell et al. 2002). Even the occurrence of three

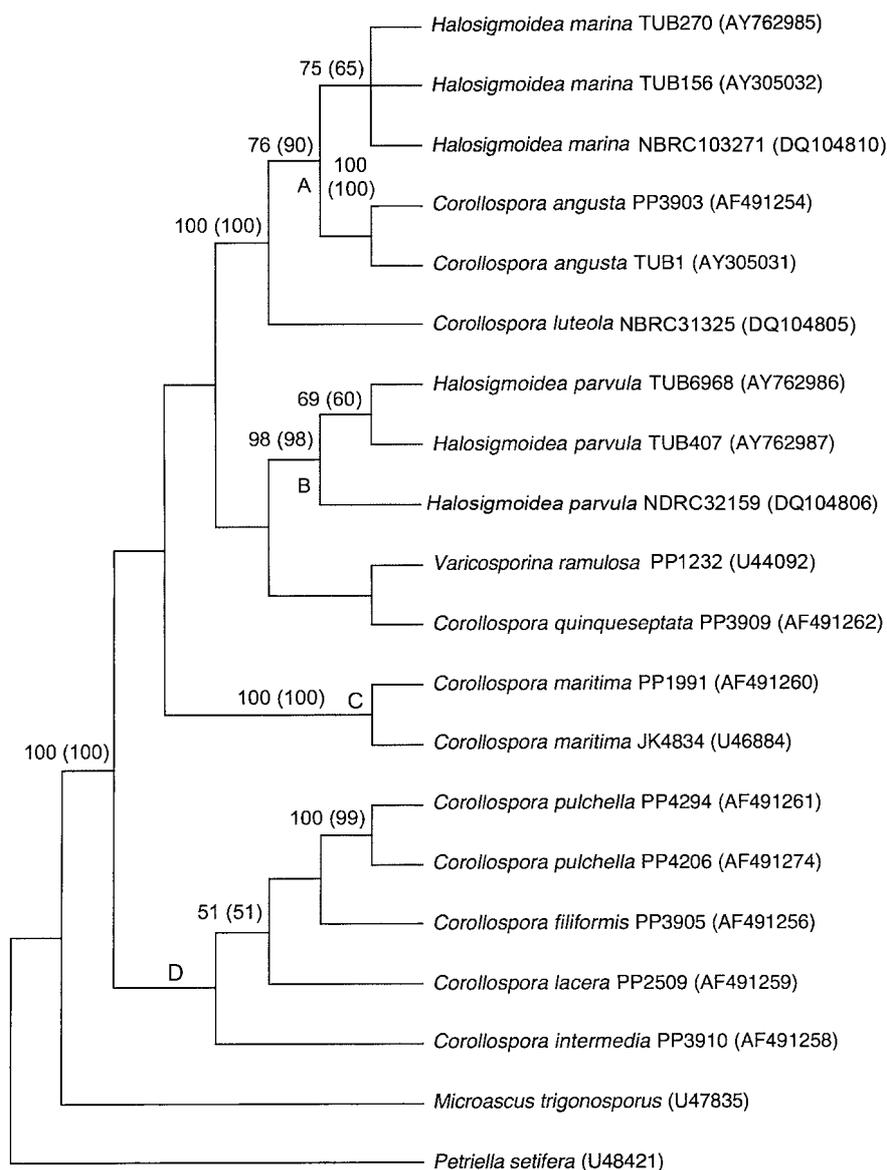


Figure 2 One of the consensus parsimonious trees inferred from LSU rDNA sequences. Bootstrap values ($\geq 50\%$) for maximum parsimony values and maximum likelihood analyses (in parentheses) are indicated above the branches.

anamorphic genera within the genus (*Clavariopsis*, *Halosigmoidea*, *Varicosporina*) does not offer the prospect of subdivision of the genus. Data presented in this study (Figure 3) suggest a possible subdivision in the genus, with two unidentified species forming a sister group to *Corollospora colossa*.

Further studies are required to resolve the phylogenetic relationship of *Corollospora* species and their anamorphs, using a wider range of taxa, duplicate strains, and more genes.

Morphological and molecular evidence indicates that the marine *Sigmoidea* species are distantly related to the freshwater species, and we propose the transfer of the marine *Sigmoidea luteola* and *Sigmoidea marina* to the new genus *Halosigmoidea*, and the description of a new species (*Halosigmoidea parvula*) to accommodate *Sigmoidea* sp. isolated from Japan (NBRC 32159, 32160, 103558) and the collections made in the Baltic Sea, Germany (407, 6582, 6968, 6989).

Taxonomy

Sigmoidea J.L. Crane

Type species *Sigmoidea prolifera* (R.H. Petersen) J.L. Crane.

MycoBank MB339266.

Freshwater anamorphic ascomycete (Dothideomycetes) with hyaline, sigmoid or curved or straight, not coiled, and many times septate conidia. Conidiogenous cells not phialidic, as repeated proliferation on at least one locus has not been demonstrated (Descals and Webster 1982).

Pseudosigmoidea Ando et Nakamura

Type species *Pseudosigmoidea cranei* Ando et Nakamura.

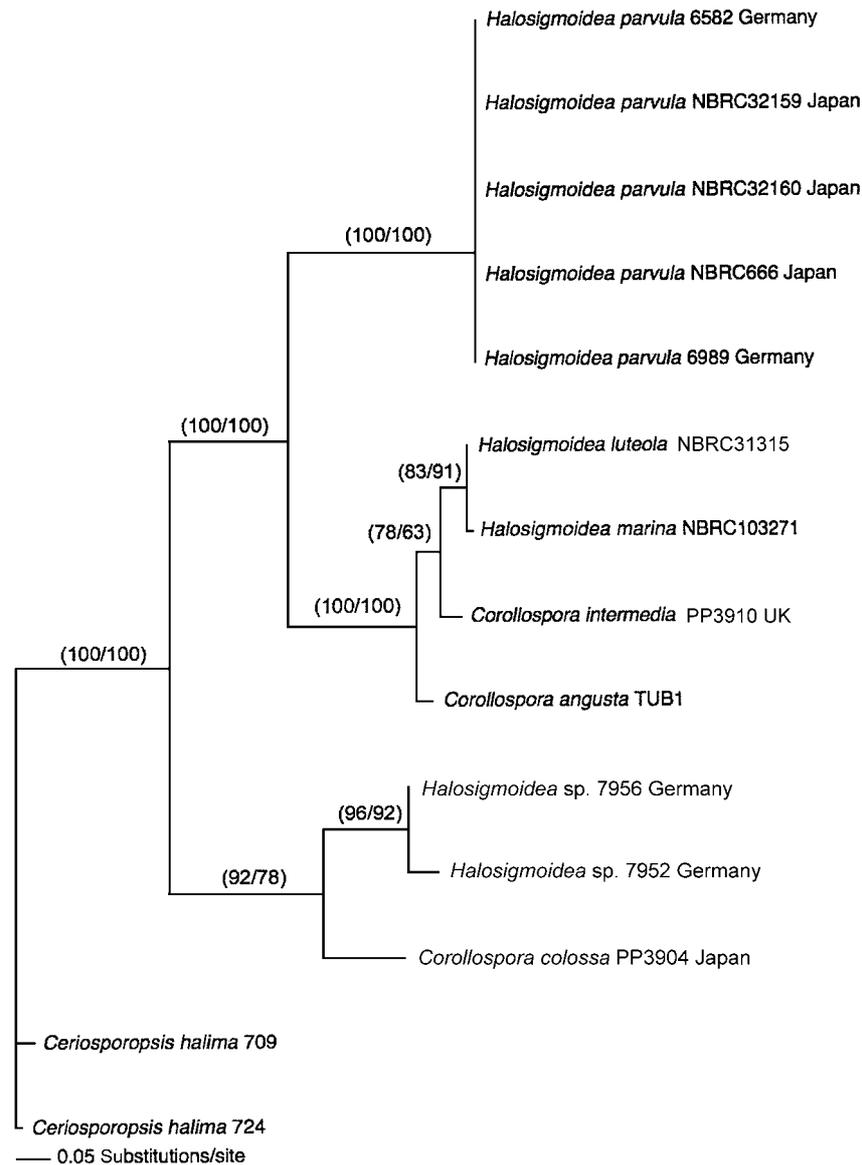


Figure 3 Maximum likelihood tree ($-L=3178.73267$) showing the relationship between *Halosigmoidea*, *Sigmoidea*, and *Corollospora* species using ITS-5.8S rDNA.

Nucleotide frequencies (A=0.214, C=0.307, G=0.28, and T=0.199), the substitution rates and shape parameter (0.546) were estimated from the dataset. Bootstrap values ($\geq 50\%$) for the maximum likelihood and parsimony (in parentheses) analyses are shown above the branches.

Table 1 Proportion of site variation between *Halosigmoidea* (*H.*) and *Corollospora* (*C.*) species for 524 aligned nucleotides, estimated using PAUP.

Fungal strains	<i>H. parvula</i> 32159	<i>H. parvula</i> 32160	<i>H. parvula</i> 666	<i>H. parvula</i> 6582	<i>H. marina</i> 103271	<i>Sigmoidea</i> sp. 7956	<i>C. angusta</i> m211
<i>H. parvula</i> 32159	–	0	0	0.0019	0.2926	0.3530	0.2809
<i>H. parvula</i> 32160		–	0	0.0019	0.2926	0.3530	0.2809
<i>H. parvula</i> 666			–	0.0019	0.2926	0.3530	0.2809
<i>H. parvula</i> 6582				–	0.2949	0.3555	0.2831
<i>H. marina</i> 103271					–	0.3271	0.0958
<i>Sigmoidea</i> sp. 7956						–	0.3407
<i>C. angusta</i> m211							–

Proportion of sites differing out of 524 sites compared (taking into account gaps).

Mycobank MB464825.

Freshwater anamorphic ascomycete (Dothideomycetes) with hyaline conidia, conidiogenesis holoblastic with rhexolytic secession of conidia, but no evidence was presented for a frill at the base of the conidium (Marvanová and Descals 1987, Ando and Nakamura 2000). The species was described as having enteroblastic polyphialdic conidiogenesis, but there is no evidence of repeated production of conidia from the same locus.

***Halosigmoidea* Nakagiri, K.L. Pang et E.B.G. Jones, gen. nov.**

Mycobank MB513326.

Marine anamorphic ascomycetes (Sordariomycetes): Mycelium ex hypis ramosis, septatis, hyalinis. Conidiophoris hyalinis, pleurogena. Cellulis conidiogenesis holoblasticis, terminalibus vel sympodialiter denticulatis. Conidiis curvati usque ad effundendam volutionem, solitariis, septatis, hyalinis. Habitat marina.

Holotypus generis *Halosigmoidea luteola* (Nakagiri et Tubaki) Nakagiri K.L. Pang et E.B.G. Jones. This is designated the type of the genus because of its confirmed teleomorph *Corollospora luteola* Nakagiri et Tubaki.

Holotype NBRC H-12292 (=TKB-F-5002).

Etymology Latin prefix 'Halos' refers to the marine habitat of the fungus.

Marine anamorphic ascomycetes (Sordariomycetes): mycelial hyphae branched, septate, hyaline. Conidiophore hyaline pleurogenous on the mycelium initially short and simple, then becoming longer and septate. Conidiogenous cells holoblastic, terminal, sympodial or irregularly sympodial and denticulate with schizolytic secession. Conidia aleuriospores, C- to U-shaped, rarely sigmoid, solitary, multiseptate, hyaline, terminal and basal cells of mature conidia devoid of cytoplasmic contents when mature. Marine habitat.

Halosigmoidea differs from *Sigmoidea* in the following way: (1) conidia C- or U-shaped, rarely sigmoid, (2) conidial cells slightly swollen, (3) conidial ends cells lacking cytoplasmic contents, (4) phylogenetically placed in the Halosphaerales, and (5) marine species found predominantly on decaying seaweeds, especially members of the Fucales (Haythorn et al. 1980, Zuccaro and Mitchell 2006). Mature conidia are generally not constricted at the septa, but before germination, each conidial cell becomes rounded and the filament separates into individual cells or several cell clusters, from which hyphae germinate.

***Halosigmoidea luteola* (Nakagiri et Tubaki) Nakagiri, K.L. Pang et E.B.G. Jones, comb. nov.**

Mycobank MB110080.

Basionym *Sigmoidea luteola* Nakagiri et Tubaki. 1982. *Trans. Mycol. Soc. Japan* 23: 102.

Hyphae branched, septate, hyaline. Conidiophore hyaline, pleurogenous on the mycelium, initially short and simple, then becoming longer, wall smooth and thin.

Conidiogenous cells holoblastic, terminal, sympodial or irregularly sympodial and denticulate schizolytic secession. Conidia aleuriospores, rarely sigmoid, solitary, septate, slightly to moderately constricted at the septa, hyaline, 106–223×5–8 µm, 7–13 (–18) septate, end cells of mature conidia devoid of cytoplasmic contents when mature and longer than the other cells in the conidium. Colonies on PDA (200 g l⁻¹ potatoes, 15 g l⁻¹ dextrose, 20 g l⁻¹ agar) with natural filtered seawater moderately to strongly yellow.

Teleomorph is *Corollospora luteola* Nakagiri et Tubaki, MycoBank: MB109694.

Material examined Japan, Tateyama-shi, Chiba Pref., ascospores isolated from sea foam, 27 February 1981. A. Nakagiri, NBRC 31315 (=ATCC 48862, TKBC-1250) ex-type *Corollospora luteola*.

***Halosigmoidea marina* (Haythorn et E.B.G. Jones) Nakagiri, K.L. Pang et E.B.G. Jones, comb. nov.**

Mycobank MB114022.

Basionym *Sigmoidea marina* Haythorn et E.B.G. Jones. 1980. *Trans. Brit. Mycol. Soc.* 74: 620.

Hyphae 2–4 µm in diameter, branched, septate, hyaline. Conidiophores hyaline pleurogenous on the mycelium initially short and simple, becoming longer and septate, wall smooth and thin. Conidiogenous cells holoblastic, terminal, sympodial or irregularly sympodial and denticulate. Conidia aleuriospores, C-shaped to slightly sigmoid, solitary, 7–11 septate, somewhat strongly constricted at the septa, hyaline, (80–) 110–180 (–231)×4–7 µm, end cells conidia devoid of cytoplasmic contents when mature and longer than the other conidial cells. Conidial secession schizolytic. Colonies on PDA (200 g l⁻¹ potatoes, 15 g l⁻¹ dextrose, 20 g l⁻¹ agar) with natural filtered seawater hyaline and pale yellow to orange with age. Teleomorph is unknown; however, molecular sequence data indicate that it should be in the genus *Corollospora*.

Material studied Ex-type NBRC 103271 (=AN-690, G. Jones PP 0423), TUB 270, and TUB 156.

Halosigmoidea marina differs from *Halosigmoidea luteola* in that: (1) conidia are more curved, (2) conidia are shorter and narrower, (3) conidia have fewer cells, (4) colonies are orange and slimy, (5) it is isolated from marine algae, and (6) has no known teleomorph.

***Halosigmoidea parvula* Zuccaro, Mitchell et Nakagiri sp. nov. (Figures 4–6)**

Mycobank MB513327.

Colonia in cultura agarosa tardo incremento, 10 mm in 7 dies expandente. Principio colonarium subalbo colore, temporis decursu colore pallente-fusco. Insensibilis odor. Copiosa sporulatio sine demensione in aqua. Compactum mycelium sine aereo mycelio. Crassae hyphae 1.5–2.5 µm plerumque immersae in medio, hyalinae, septatae, ramosae, non costrictae ad septa. Interdum anasto-

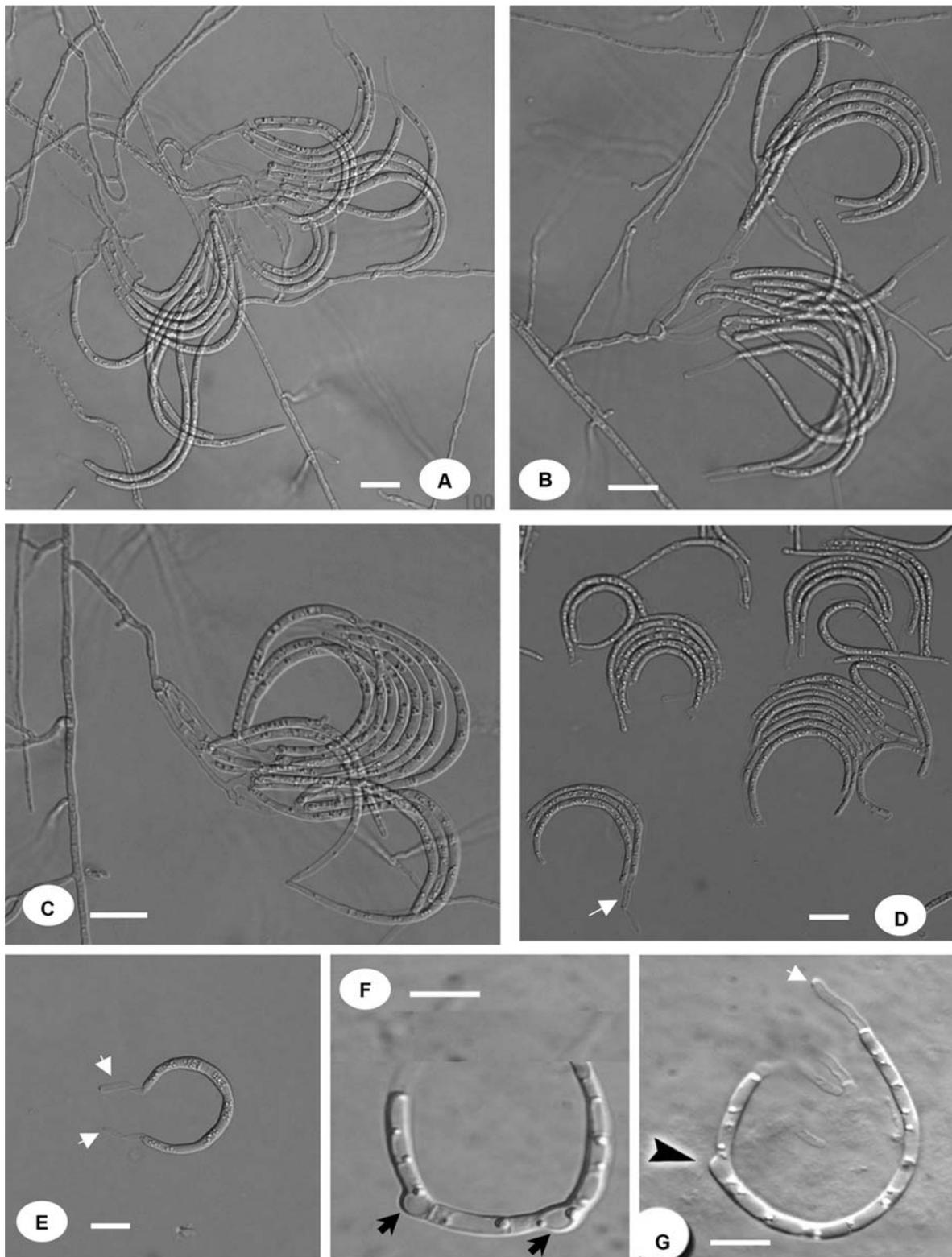


Figure 4 *Halosigmoidea parvula*.

(A, C, D) Conidiophores bearing conidiogenous cells. (B, E–G) Conidia with empty end cells. Protrudence at septa arrowed. Scale bars: 10 μm .

mosae adsunt. Conidiophori longi 2.0–60 μm , lati 2–3 μm , inramosi vel paulum ramosi, plerumque longi 20 μm , cum inramosi, hyalini, paulum septati, pleurogeni, cum tenue pariete sunt. Cellula conidiogena holoblastica terminalis, monoblastica, sympodialis cum denticulis prominentibus vel nonnumquam pariete polita. Conidii (32–

85–125 (–140) μm longi 3–5 μm crassi, solitari, filiformes, curvati usque ad efficaciam volutionem cum 4–8 (–10) septis, plerumque 5 constrictis ad septa, sed interdum. Conidiorum cellulis finem facentibus quandoque deest quod in iis inest; illae obtusae altera base et filiformes altera extrema parte sunt, aut utraque extrema



Figure 5 *Halosigmoidea parvula*. (A, B) Branched conidiophores with terminal conidiogenous cells. (C) Inset of conidiogenous cells. Scale bars: 10 μm .

pars filiformis vel utraque obtusa est. Teleomorphus ignota.

Holotypus subspecie *coloniae* in *Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands) depositus (CBS number 114753)*. *Locus classicus* in *mare Baltico, Germania; holotypus (TUB 6989) e planta aquatica Zostera marina mense Martio 2003 separatus est.*

Colonies slow-growing on PDA with natural filtered seawater, 10 mm diameter within 7 days at 20–25°C, 3 mm diameter within 7 days at 14°C in the dark. Mycelium on glucose-yeast extract agar medium colorless at first, later pale brown to pale yellow, compact with little aerial mycelium. Conidia are produced in abundance without submergence in water. Hyphae 1.5–2.5 μm in diameter, mostly immersed, hyaline, septate, branched, and not constricted at the septa. Anastomoses sometimes present. Conidiophores 2.0–6.0 \times 2–3 μm , simple or sparsely branched, pleurogenous on the mycelium, typically 20 μm long when unbranched, hyaline, cells one to few, walls smooth and thin (Figures 4A, B and 5A, B). Conidiogenous cells holoblastic, acrogenous, irregularly sympodial, often denticulate at the apex (Figure 5 A–C).

Conidia (32–) 85–125 (–140) \times 3–5 μm (including the ends lacking cytoplasm when present), solitary or in groups, filiform, curved, C- or U-shaped, 4–8 (–10) septate, typically with 5 middle cells slightly constricted at the septa. The terminal cells of mature conidia are sometimes devoid of cytoplasm; the proximal end is mostly obtuse and the distal end filiform, both ends are rarely obtuse or both filiform (Figure 4B, C, E–G). Teleomorph unknown. Mode of life: saprophytic. Substrate: *Polysiphonia* sp. and *Ceramium* sp. (Rhodophyta) and *Zostera marina*.

On media without salt, older mycelium is torulose with chlamydo-spore-like structures and becomes more darkly pigmented. Young hyphae are 3–4 μm , older up to 9 μm wide, no aerial mycelium is present. Sporulation rare, with conidia irregularly swollen. Growth is 5 mm diameter week⁻¹ at 20–25°C dark.

Holotype A culture derived from type (TUB 6989) has been deposited at the *Centraalbureau voor Schimmelcultures (Utrecht, Netherlands)* with the CBS number 114753.

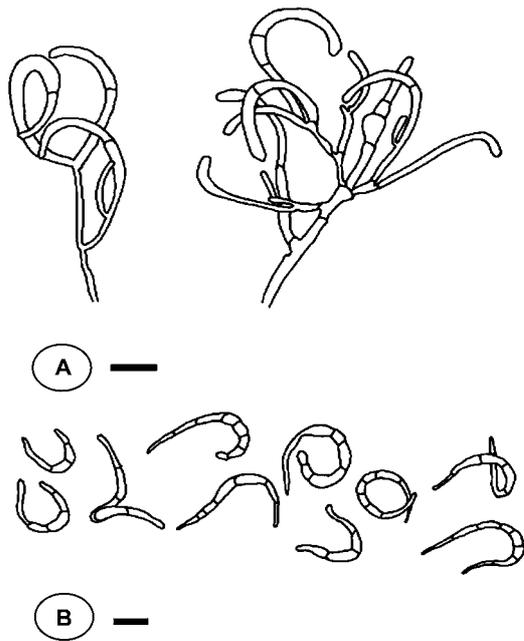


Figure 6 *Halosigmoidea parvula*: line drawings. (A) Conidiophores and conidia. (B) Conidia. Scale bars: 10 μm .

Material examined Germany, Wismar Bucht, *Zostera marina*, March 2003, A. Zuccaro, TUB 6989 (=CBS 114753); Ahrenshoop, *Polysiphonia* sp. September 2002, A. Zuccaro, TUB 6582; Travemunde, *Ceramium* sp. May 2004, A. Zuccaro, TUB 407. Japan, Ohya, Motoyoshi-cho, Miyagi Pref., sea foam, 29 October 1981, A. Nakagiri, NBRC 32159 (=AN-708); Hamanaka, Horoto, Hokkaido, sea foam, 30 Aug. 1984, A. Nakagiri, NBRC 32160 (=AN-931); and Sarufutsu, Soya-gun, Hokkaido, sea foam, 22 Aug. 1981, NBRC 103558 (=AN-666).

Key to genera previously assigned to *Sigmoidea* J.L. Crane

1. Conidiogenous cells “polyphialidic” enteroblastic.....
.....*Pseudosigmoidea*
1. Conidiogenous cells sympodial, holoblastic and denticulate.....2
 2. Conidial end cells lacking cytoplasmic contents, conidia C- to U-shaped, colonies colored and slimy, marine.....*Halosigmoidea*
 2. Conidia sigmoid, end cells with contents, freshwater.....*Sigmoidea*.

Key to *Halosigmoidea* species

1. Colony bright yellow, conidia 106–223 μm long, 5–8 μm wide near the middle, 7–13 (–18) septate
.....*Halosigmoidea luteola*
1. Colony hyaline to pale yellow with age2
 2. Conidia (32–) 85–125 (–140) μm long, 3–5 μm wide near the middle, 4–8 (–10) septate
.....*Halosigmoidea parvula*
 2. Conidia (80–) 110–180 (–231) μm long, 4–7 μm wide near the middle, 7–11 septate
.....*Halosigmoidea marina*.

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