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Koralionastetales, a new order of marine *Ascomycota* in the *Sordariomycetes*

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ABSTRACT

Based on molecular studies using 1760 bp of the nuSSU and 604 bp of the nuLSU rRNA genes and using morphological characters, the genera *Koralionastes* and *Pontogeneia* are assigned to the new order *Koralionastetales*, family *Koralionastetaceae*, class *Sordariomycetes*. *Koralionastetales* is a sister group to *Lulworthiales*; differences in morphological characters are expressed in the ascospores and the presence/absence of periphyses and paraphyses. A new species of *Pontogeneia*, *P. microdictyi* from *Microdictyon* sp. in the Bahamas, is described.

Introduction

The genus *Pontogeneia* was described by Kohlmeyer (1975) for five perithecial ascomycetes parasitizing marine *Phaeophyta*. Two more species have been added since (Kohlmeyer & Kohlmeyer 1979; Kohlmeyer & Demoulin 1981), and a new species is described in the present paper. The family *Koralionastetaceae* was proposed by Kohlmeyer & Volkmann-Kohlmeyer (1987) based on the single genus, *Koralionastes*, with three species from corals. Two species were added later by Kohlmeyer & Volkmann-Kohlmeyer (1990). The proper placement of both *Pontogeneia* and *Koralionastes* in the fungal kingdom was unclear until now. In *Outline of Ascomycota* (Eriksson 2006), *Pontogeneia* was placed among the genera of uncertain position in the *Sordariomycetes*, and *Koralionastes* (*Koralionastetaceae*) was listed among families and genera of *Ascomycota* that cannot be placed in any accepted class and order. The

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present molecular investigation was initiated to clarify the phylogenetic position of these two genera.

Materials and methods

Isolates and herbarium material

Koralionastes ascomata used in this study were found on coral rocks embedded in yellow, leathery, well-delimited crusts of sponges (Kohlmeyer & Volkmann-Kohlmeyer 1987). Collected rocks were maintained in buckets of seawater until microscopic examination at a field station, the same day or the day after. Ascomata were numbered, and the contents of each ascoma were preserved as a permanent slide and in DNA extraction buffer. Permanent slides were prepared according to Volkmann-Kohlmeyer & Kohlmeyer (1996), mounted using Fisher Scientific Permount Mounting Medium (Fisher Scientific Research, Pittsburgh, PA). DNA lysis buffer consisted of 150 mM EDTA, 50 mM Tris–HCl (pH 8), 1 % SDS (Sambrook *et al.* 1989). Ascospores were stored in 50 µl DNA lysis buffer in Parafilm-sealed 1.5 ml Eppendorf tubes.

Because of their highly specialized life style, members of *Koralionastes* are difficult to collect and would not grow on culture media despite repeated attempts. No fresh material of *Pontogeneia* was obtainable. Therefore, only small collections were available and DNA extraction had to be made from the contents of dried or preserved ascomata, except for the *Koralionastes* collections (J.K. 5769, J.K. 5771) described above. Voucher material of the fungi on which this paper is based has been permanently preserved at MycoBank, IMS and/or NY.

Material examined: *Koralionastes ellipticus:* **Belize**: Carrie Bow Cay, East side, on coral rocks with sponges; 19 Nov 2004; *P. Inderbitzin and X. Fang* [J.K. 5769 (PC35); J.K. 5771 (PC305, PC306)] (IMS). *Pontogeneia microdictyi*: see under Taxonomy. *Pontogeneia erikae*; **USA**: *California*, San Diego County, Encinitas, on *Egregia menziesii*; 10 Aug 1979; *J. Kohlmeyer* [J.K. 4015] (IMS—isotype); same location and host; 12 Aug 1979. *J. Kohlmeyer* [J.K. 4100] (IMS). *Pontogeneia padinae*: **Mexico**: *Prov. Sonora,* Puerto Peñasco, on *Padina durvillaei*; 22 June 1974; *J. Kohlmeyer* [J.K. 3541](IMS—isotype).

DNA extraction, amplification, cloning and sequencing

A standard phenol–chloroform protocol (Lee & Taylor 1990) as described in Inderbitzin *et al.* (2004), was used to extract DNA from seven pooled *Koralionastes ellipticus* ascomata contents that were designated as sample PC35, and approximately 30 pooled, air-dried ascomata of *Pontogeneia microdictyi* referred to as sample P415. A modified protocol of Raja *et al.* (2003) was used to extract DNA from single *K. ellipticus* ascomata designated as samples PC305 and PC306. The ascomal contents were removed by cutting the ascoma open, dropping sterile water from the tip of a needle on the ascoma, and then removing the contents with the needle and transferring to 400 µl AP1 buffer (DNeasy Plant Mini Kit, Qiagen Inc., Valencia, CA). This was incubated with 200 U lyticase (Sigma-Aldrich, St Louis, MO) at 30 °C for 4 h and ground at hourly intervals during incubation using a sterile micropestle. RNase A (40 mg; Qiagen Inc., Valencia, CA) was added and incubated for 20 min at 65 °C. The protocol was then followed from step 3 of the DNeasy Plant Mini Kit (Qiagen Inc., Valencia, CA).

The LSU region of the rDNA was amplified using the LROR to LR6 primers (Vilgalys & Hester 1990) with a Taq PCR Core Kit (Qiagen Inc., Valencia, CA) following the PCR cycling parameters of Vilgalys & Hester (1990). The SSU region of the rDNA was amplified using primer pair sets: NS1–NS4 and NS5–NS8 (White *et al.* 1990); SL1 (Landvik 1996) and NS2 (White *et al.* 1990); or NS23 (Gargas & Taylor 1992) and NS6 (White *et al.* 1990) following the PCR cycling parameters of Anderson *et al.* (2001). The PCR products were purified using QIAquick PCR purification kit (Qiagen Inc., Valencia, CA) according to the manufacturer's instructions. Amplification specificity was confirmed using gel electrophoresis and PCR products were quantified using an ND-1000 spectrophotometer (NanoDrop, Wilmington, DE).

Samples yielding low DNA concentrations were cloned following manufacturer's instructions using either a pGEM-T Easy Vector cloning kit (Promega Corp., Madison, WI) or a Topo TA Cloning Kit with a pCR2.1-TOPO Vector and One Shot TOP10 Chemically Competent Cells (Invitrogen Corp., Carlsbad, CA). Transformants were directly amplified using the Taq PCR Core Kit (Qiagen Inc., Valencia, CA) as follows: initial denaturing step of 2 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 20 s at 50 °C and 1.5 min at 72 °C, with a final extension of 7 min at 72 °C.

Sequencing of the PCR products and the clones was performed at either the Champaign Core Sequencing Facility, University of Illinois at Urbana-Champaign, or at the Cornell University Life Sciences Core Laboratories Center on an Applied Biosystems Automated 3730 DNA Analyser using Big Dye Terminator chemistry and AmpliTaq-FS DNA polymerase (Applied Biosystems, Foster City, CA).

The specific primer P35f (5′-GAGGGTTAGGGCTCGACC-3′) was designed based on the cloned SL1/NS2 sequence of *K. ellipticus* sample PC35, and used together with primer cITS5, the complement of ITS5 (White *et al.* 1990) in a PCR amplification. The PCR product was sequenced for complete coverage in both directions with forward primers PC35f, PC35fi (5′-GTTAGGGCTCGACCCCG GAG-3′), Bas3 and MB1 (Inderbitzin *et al.* 2001), PC35f5 (5′-GAG GATTGACAGTTTGAGAGC-3′) and reverse primers cITS5i (5′-GTTACGACTTTTACTTCCTCTA-3′), cITS5, MB2, and cBas3 (Inderbitzin *et al.* 2001). Sequencing coverage of the SSU of *P. microdictyi* sample P415 was obtained with specific primers designed based on a NS23/NS6 amplicon. For 5′-end coverage, specific reverse primer Ps1r (5′-AGCCGGCGTGCCGCCAGG-3′) was used with primer SL1 in a PCR reaction, and sequenced in both directions with primers SL1, Pof (5′-GCCCGTAGTT GAACCTTG-3′), Bas3 and Por (5′-CACGGTAAGCGCTACAAG-3′), cBas3 and NS2. At the SSU 3′-end, specific forward primer Ps1f (5′-CCTGGCGGCACGCCGGCT-3′) was used with NS8 (White *et al.* 1990) for PCR and sequencing.

Phylogenetic analyses

Sequences were aligned with published sequence data (Table 1) using Clustal-X (Thompson *et al.* 1997), and then refined manually in Se-Al (Rambaut 1996). The rationale for taxon sampling was based on Campbell *et al.* (2005) and Zhang *et al.* (2006).

Table 1 – Fungal species, source code, and GenBank accession numbers included in the analyses

Taxon	Source[a]	GenBank accession number	
		SSU	LSU
Aniptodera chesapeakensis	ATCC 32818[b]	U46870	U46882
Bionectria ochroleuca	IAM 14569/CCFC 226708	AB003950	AY283558
Botryotinia fuckeliana	AFTOL-ID 59	AY544695	AY544651
Colletotrichum gloeosporoides	N/A	M55640	Z18999
Diatrype disciformis	AFTOL-ID 927	DQ471012	DQ470964
Eutypa lata	AFTOL-ID 929	DQ836896	DQ836903
Glomerella cingulata	FAU 513	U48427	U48428
Haloguignardia irritans	N/A	AY566252	N/A
Halosphaeria appendiculata	CBS 197.60	U46872	U46885
Hypocrea citrina	OSC 100005	AY544693	AY544649
Kohlmeyeriella crassa	IFO 32133[b]	AY879005	AY878963
K. crassa	IFO 32134	AY879006	AY878964
K. tubulata	PP 0989	AY878997	AF491264
K. tubulata	PP 1105	AY878998	AF491265
Koralionastes angustus	**JK 5366**	**failed**	**failed**
K. angustus	**JK 5367**	**failed**	**failed**
K. ellipticus	**PC 310**	**failed**	**failed**
K. ellipticus	**JK 5771 (PC 305)**	**EU863580**	**EU863583**
K. ellipticus	**JK 5771 (PC 306)**	N/A	**EU863584**
K. ellipticus	**PC 311**	**failed**	**failed**
K. ellipticus	**JK 5199**	**failed**	**failed**
K. ellipticus	**JK 5769 (PC 35)**	**EU863581**	**EU863585**
K. giganteus	**JK 5354**	**failed**	**failed**
K. ovalis	**JK 5277**[b]	**failed**	**failed**
K. ovalis	**JK 5281**	**failed**	**failed**
Leotia lubrica	AFTOL-ID 1	AY544687	AY544644
Lindra crassa	ATCC 56663	AY878999	N/A
L. obtusa	IFO 31317[b]	AY879002	AY878960
L. obtusa	CBS 113030	AY879001	AY878959
L. thalassiae	JK 4743	AF047580	AF047581
L. thalassiae	JK 4322A	AF195632	AF195633
Lulwoana uniseptata	IFO 32137[b]	AY879031	AY878988
L. uniseptata	PP 4032	AY879032	AY878989
Lulwoidea lignoarenaria	IFO 32135	AY879010	AY878968
L. lignoarenaria	ATCC 64644	AY879009	AF491272
Lulworthia fucicola	ATCC 64288[b]	AY879007	AY878965
L. fucicola	PP 1235	N/A	AF491270
L. grandispora	JK 4686	AF047582	AF047583
L. grandispora	JK 5168A	AY879012	AY878969
L. medusa	JK 5581A	AF195636	AF195637
L. cfr. *opaca*	CBS 218.60	AY879003	AY878961
L. cfr. *purpurea*	CBS 219.60	AY879004	AY878962
Microascus trigonosporus	RSA 1942	L36987	U47835
Microglossum rufum	AFTOL-ID 1292	DQ471033	DQ470981
Petriella setifera	ATCC 26490	U32421	U48421
Pontogeneia erikae	**JK 4015**	**failed**	**failed**
P. padinae	**JK 3541**[b]	**failed**	**failed**
P. microdictyi	**JK 5747**	**failed**	**failed**
P. microdictyi	**JK 5748 (PC 415)**	**EU863582**	N/A
Spathulospora adelpha	JK 5599	AY380314	AY380314
S. antarctica	JK 3530	AY380315	AY380315
Xylaria curta	JDR 42375	U32417	U47840
X. hypoxylon	ATCC 42768	U20378	U47841

Taxa sequenced for this study are in boldface.

a AFTOL, Assembling the Fungal Tree of Life; ATCC, American Type Culture Collection; CBS, Centraalbureau voor Schimmelcultures; FAU, culture collection of F.A. Uecker; IAM, Institute of Applied Microbiology, University of Tokyo, Japan; IFO, Institute for Fermentation, Osaka, now NBRC, NITE Biological Resource Center, Chiba; JDR, culture collection of J.D. Rogers, Washington State University; JK, Herb. J. Kohlmeyer (IMS); OSC, Oregon State University Herbarium; PC, Phabagen Culture Collection, University of Utrecht, Netherlands; PP, University of Portsmouth, Culture Collection of Marine Fungi; RSA, Rancho Santa Ana culture collection; N/A, not available.

b Ex-type strains.

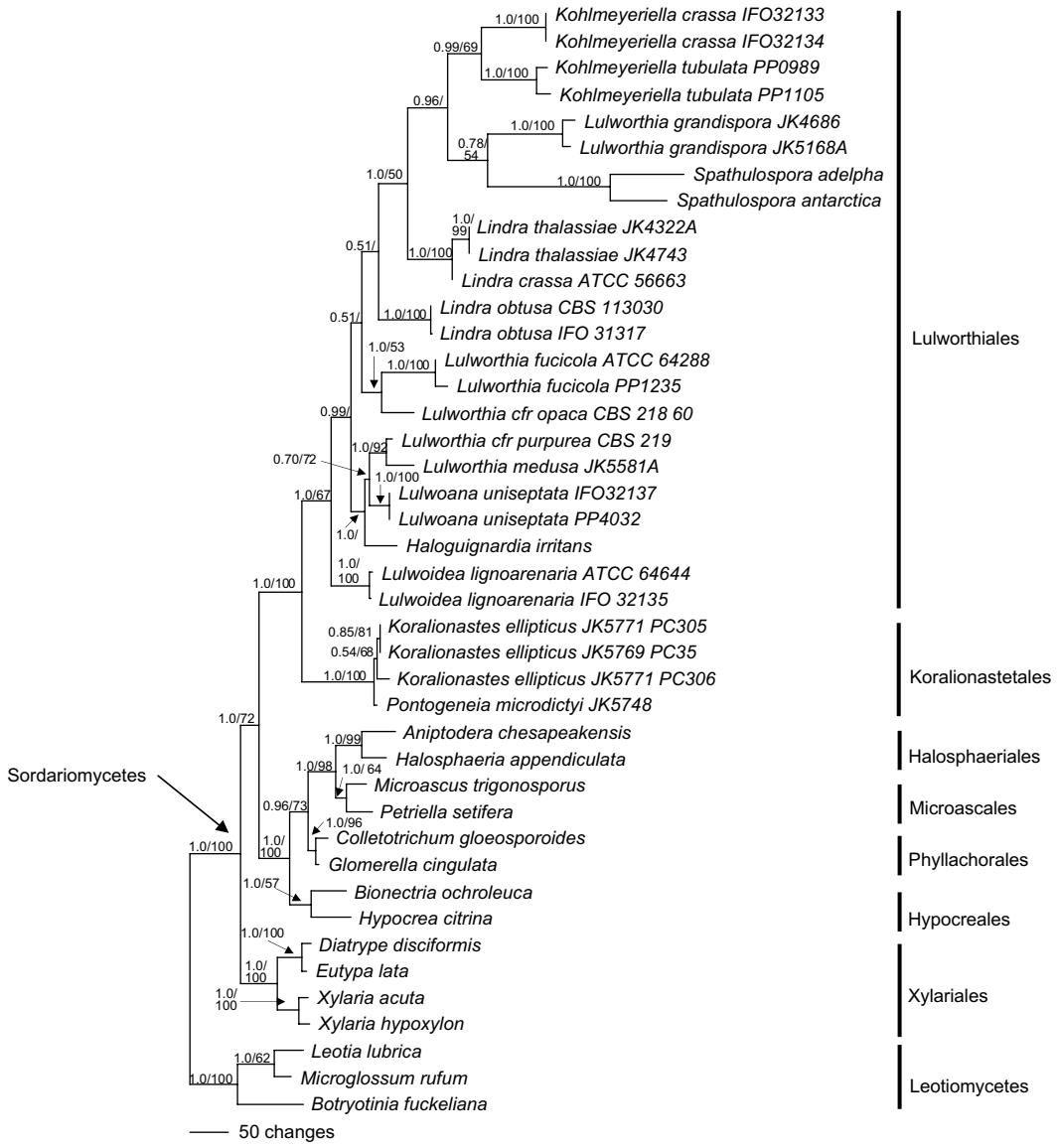


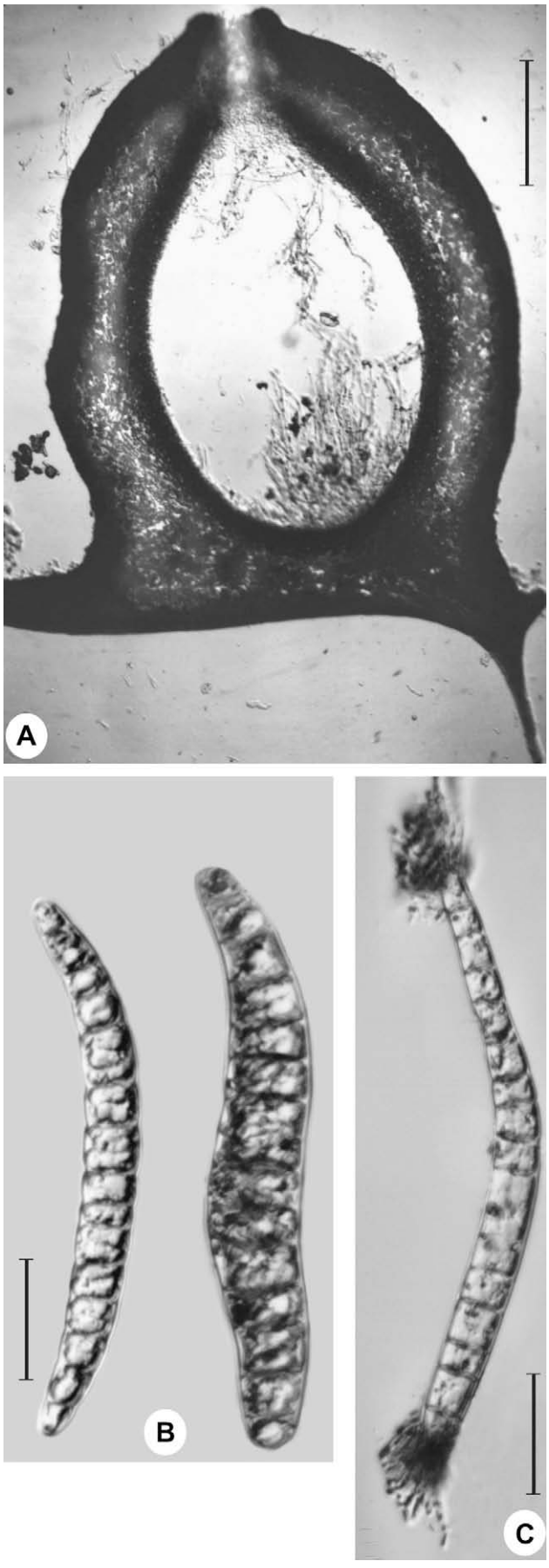
Fig 1 – B-MCMCMC consensus tree from 19 570 trees inferred from Bayesian analyses of the combined 18S and 28S rDNA data. PP values and MP BS values greater than 50 % are given, respectively, at the corresponding nodes.

Incongruence between datasets was tested using a 70 % reciprocal MP BS comparison (Mason-Gamer & Kellogg 1996). Bayesian Metropolis-coupled MCMC (B-MCMCMC) analyses were performed with the GTR + I + Γ model using MrBayes 3.0 (Huelsenbeck & Ronquist 2001). Searches were conducted for a total of 2 M generations on four chains with phylogenetic trees sampled every 100 generations. Three independent analyses were conducted to verify likelihood convergence and burn-in parameter.

MP analyses of the combined SSU and LSU rDNA sequence data were performed using PAUP version 4.0b 10 (Swofford 2002). Heuristic searches were run employing random starting trees, random stepwise addition on 100 replicates and a tree-bisection–reconnection branch-swapping algorithm. Weighted parsimony (WP) analyses were performed using a step matrix to weight nucleotide transformations based on the reciprocal of the observed transition: transversion (ti:tv) ratio (Spatafora *et al.* 1998). Gaps were treated as missing data.

ML analyses were run in PAUP using the GTR + I + Γ model, as determined by ModelTest (Posada & Crandall 1998). Analyses were performed with heuristic searches, random starting trees with as-is addition of taxa, and a tree-bisection–reconnection branch-swapping algorithm.

The different tree topologies were compared using Kishino–Hasegawa (K–H) (Kishino & Hasegawa 1989) and Shimodaira–Hasegawa (S-H) (Shimodaira & Hasegawa 1999) ML tests, implemented in PAUP, to determine if any of the trees were significantly worse than each other. Nodal support was assessed by non-parametric bootstrapping and Bayesian PPs. BS values (Felsenstein 1985) were calculated from 1 K replications using a heuristic search on 100 replicates with random starting trees, random stepwise addition and MulTrees off.



PPs were calculated from the majority rule consensus tree of the 20 K trees from the Bayesian analyses, with the initial 430 trees excluded as burn-in prior to the convergence of likelihoods.

Results

There were 11 collections representing four species of *Koralionastes*, and four collections representing three species of *Pontogeneia* available for sequencing (Table 1). Unfortunately, DNA was successfully extracted, amplified, cloned and sequenced from only three collections of *Koralionastes* representing one species, and from only one collection, i.e. species, of *Pontogeneia*. Both the SSU (1760 bp) and LSU (604 bp) rDNA were sequenced from three of those four collections; only the LSU rDNA was sequenced from one collection as there was insufficient DNA yield to also sequence the SSU rDNA.

Of 2392 unambiguously aligned characters, 708 were parsimony informative. The reciprocal comparisons of the 70 % BS trees did not reveal any incongruence. The heuristic searches in MP analyses generated two most parsimonious trees (MPT), the WP analyses generated one MPT; all three were 2565 steps in length. The ML tree was one step longer at 2566. These were compared with the Bayesian tree with the lowest –ln L (Fig 1). Both K–H and S–H likelihood tests indicated one of the two unweighted MPTs was significantly worse than all the other trees. There was no significant difference among the other trees.

The topologies of the Bayesian, weighted, ML and one of the unweighted trees were identical. The second unweighted tree, which was significantly worse in the S–H and K–H tests, differed from the rest of the trees only by the placement of *Lindra obtusa*, which was in a clade with *Lulwoana uniseptata*.

In all topologies *Lulworthiales* formed a monophyletic clade. The taxa of *Koralionastes* and *Pontogeneia* formed a monophyletic clade basal to the clade of *Lulworthiales*. The separation of this clade from the clade of *Lulworthiales* was supported with a PP of 1 and a MP BS of 100 %.

Taxonomy

Koralionastetales Kohlm., Volkm.-Kohlm., J. Campb. & Inderbitzin, **ord. nov.**
MycoBank no.: MB 512605

Ab ordinibus ceteris Sordariomycetes differt origine antheridiorum in ascosporis germinantibus, absentia annulorum apicalium; semper marinus, cum coralliis et spongiis consotiatus, vel parasiticus in algis.

Fig 2 – *Pontogeneia microdictyi*. (A) Longitudinal section (16 μm) through ascoma. (B) Ascospores. (C) Germinating ascospore; antheridia developing on germ tubes. (A) Under brightfield, (B–C) under Nomarski interference contrast microscopy. (A) From J.K. 5695, (B–C) from J.K. 5674. Bar = (A) 200 μm; (B–C) 50 μm.

Table 2 – Morphological characters and hosts of *Pontogeneia* species

Species	Ascospore Size (μm)	Septa	Peridium Thickness	Hosts	Host Phyla
Pontogeneia. microdictyi	200–275 × 19–26	9–18	75–160	*Microdictyon* sp.	Chlorophyta
P. calospora	(54–) 62–85 × 8–12 (–14)	3–5 (–6)	40–60	*Castagnea chordariaeformis*	Phaeophyta
P. codiicola	52–79 × 20–28	3	16–26	*Codium fragile, C. simulans*	Chlorophyta
P. cubensis	215–325 × 13.5–17 (–21)	(10–) 12–13	60–80	*Halopteris scoparia*	Phaeophyta
P. enormis	280–350 × 12–14	4–5	50–80	*H. scoparia*	Phaeophyta
P. erikae	(18–) 19–26 (–28) × 8–10.5)	2	10–24	*Ectocarpus* (?)	Phaeophyta
P. padinae	99–167 (–172) × 10–12	6–9 (–10)	32–52	*Padina durvillaei*	Phaeophyta
P. valoniopsidis	72–100 × 21–30	2–5	?	*Valoniopsis pachynema*	Chlorophyta

Ascomata subglobose, ovoid or ellipsoidal, superficial, ostiolate, papillate or epapillate, periphysate, black, subiculate or without subiculum. *Hamathecium* composed of paraphyses. *Asci* eight-spored, clavate to ellipsoidal or fusiform, pedunculate, unitunicate, deliquescent, basal. *Ascospores* ellipsoidal to fusiform, multiseptate near the apices, or filiform and evenly multiseptate, hyaline, thick- or thin-walled, without apical mucus-filled chambers or gelatinous sheaths. *Antheridia* formed on germ tubes. *Spermatia* enteroblastic, subglobose.

Type family: *Koralionastetaceae* Kohlm. & Volkm.-Kohlm.

Pontogeneia microdictyi Kohlm.& Volkm.-Kohlm., **sp. nov**. (Fig 2)
MycoBank no.: MB 512078
Etym.: from the generic name of the host plant.

A speciebus ceteris Pontogeneiae differt combinatione characterum, peridio crassiore ascocarpi, et hospite *Microdictyon*.

Typus: **Bahamas**: *Sweetings Cay*, on thalli of *Microdictyon* sp., 28 July 1999, *J. Long* [J.K. 5679] (IMS—holotypus; NY—isotypus).

Ascomata 480–890 μm high, 310–825 μm in diam, subglobose to ellipsoidal, with a flat base, superficial, attached with a subiculum to the host, ostiolate, papillate or epapillate, coriaceous, black, gregarious (Fig 2A). *Papilla,* when present, about 80 μm long, 80 μm in diam, cylindrical, ostiolar canal lined with periphyses. *Peridium* 75–160 μm thick, composed of three layers, an outer plectenchymatic, dark brown layer of small cells with narrow lumina, a middle dark brown layer of larger cells with large lumina, and an inner layer of hyaline, small, flat cells, forming a *textura angularis* in longitudinal section. *Hamathecium* composed of septate, unbranched paraphyses, 4–10 μm in diam. *Asci* 250–340 × 75–90 μm, eight-spored, ellipsoidal, with a small stalk, unitunicate, not blue in IKI, developing at the bottom of the locule. *Ascospores* 200–275 × 19–26 μm ($\bar{x} = 227 \times 21$ μm, $n = 24$), filiform, straight or slightly curved, 9–18-septate (predominantly 11–13-septate), not or slightly constricted at the septa, rounded at the apices, hyaline (Fig 2B). Antheridia developing on apical germ tubes of ascospores (Fig 2C). *Hyphae* grow within cell walls of the host and are also attached to the surface of *Mycrodictyon* cells.

Host: Microdictyon sp. (*Chlorophyta*).

Distribution: Caribbean (Bahamas).

Additional specimens examined: Pontogeneia microdictyi (all on *Microdictyon* sp): **Bahamas**: *San Salvador Island*, 3 Aug 2000, *J. Long* [J.K. 5674](IMS); *Sweetings Cay*, 28 July 1999, *J. Long* [J.K. 5679](IMS); *Cay Santo Domingo*, 4 Aug 2000, *P. Jensen* [J.K. 5682](IMS); *Cat Island,* 8 Mar 2001, 20 July 2003, *P. Jensen* [J.K. 5693, 5747](IMS); *Little San Salvador Island*, 10 Mar 2001, *P. Jensen* [J.K. 5694](IMS); *Cat Cay*, 7 Aug 2000, *J. Long* [J.K. 5695](IMS); Stirrup Cay, 14 July 2003, *P. Jensen* [J.K.5748 P415] (IMS).

Discussion

Specimens of the three species of *Koralionastes* and one species of *Pontogeneia* that were sequenced are placed in a monophyletic clade basal to the clade of *Lulworthiales* (Fig 1). This clade represents a distinct taxonomic entity at the order level based on both molecular and morphological data. The DNA sequence variation within *Koralionastes*, between *K. ellipticus* samples

Table 3 – Distinction between *Koralionastetales* and *Lulworthiales*

	Koralionastetales		*Lulworthiales*
Genus	*Koralionastes*	*Pontogeneia*	*Lulworthia/Lindra*
Hamathecium	Septate paraphyses	Septate paraphyses	Absent (centrum initially filled with a pseudoparenchymatous tissue)
Periphyses	Present	Present	Absent
Ascospores	Without apical structures	Without apical structures	Mucus-containing apical chambers (exception: Lindra)
Hosts/Substrates	Coral rock; associated with crustose sponges and possibly bacteria	Algae	Mostly wood; also *Spartina, Thalassia, Zostera*, algae, coral rock

PC305 and PC306 from collection J.K. 5771, is more likely caused by DNA sequencing errors due to difficult template, as well as cloning errors, than to genuine biological variation.

Although *Koralionastes* and *Pontogeneia* are closely related to members of *Lulworthiales* (Fig 1), there are also important morphological differences (Table 3). Young ascomata of all members of *Lulworthiales* are filled with pseudoparenchyma composed of thin-walled cells that is pushed aside by developing asci; there is no hamathecium in mature ascomata and periphyses are also absent, whereas ascomata of *Koralionastes* and *Pontogeneia* have distinct paraphyses and periphyses. Furthermore, species of *Lulworthiales* have ascospores with apical mucus-containing chambers or gelatinous sheaths, excluding *Lindra* spp., which do not have apical ascospore structures, except for *L. inflata* that has apical gelatinous appendages. Ascospores of all *Koralionastes* and *Pontogeneia* species are devoid of any such structures.

Koralionastetales differs from the other orders of the *Sordariomycetes* (Zhang *et al.* 2006) by the characteristic formation of antheridia on germinating ascospores. Moreover, *Koralionastetales* can be distinguished from most of the *Sordariomycetes* orders by the absence of apical rings in the asci. Exceptions are the *Melanosporales*, *Microascales*, *Ophiostomatales,* and *Halosphaeriales*, but a combination of other characters, e.g. pseudoparenchyma *versus* paraphyses or lack of interascal tissue, etc., clearly separate them from the *Koralionastetales*. Although Hibbett *et al.* (2007) included the *Halosphaeriales* in the *Microascales*, we prefer to keep them separate as greater taxon sampling is needed for a taxonomic revision (Zhang *et al.* 2006). There is also a significant difference in life style between *Koralionastetales* and the other orders; all its members are obligately marine and are always associated with corals and sponges or algae.

Based on the phylogenetic analyses and supported by their distinctive morphology, the transfer of *Koralionastes* and *Pontogeneia* to a new order in the class *Sordariomycetes* is warranted.

The genus *Pontogeneia* includes at present seven species; all associated with marine algae, belonging to the *Chlorophyta* or *Phaeophyta* (Kohlmeyer & Kohlmeyer 1979; Kohlmeyer & Demoulin 1981). Representatives of *Pontogeneia* are rarely found; *P. cubensis, P. enormis, P. padinae,* and *P. valoniopsidis* are known only from one collection each. The new species, *P. microdictyi*, is more common and can be clearly differentiated from the other species by morphological characters (Table 2). It grows on *Microdictyon* and is most closely related to *P. cubensis* on *Halopteris*. Ascospore lengths overlap between the two species, but the diameter is usually larger in *P. microdictyi*. Whereas *P. cubensis* has up to 13 septa, there are up to 18 septa in *P. microdictyi*. The greatest difference is in the peridium, which is three-layered and 75–160 μm thick in *P. microdictyi,* but two-layered and 60–80 μm thick in *P. cubensis*. Another important difference is that the hosts belong to different phyla: green algae in *P. microdictyi*, and brown algae in *P. cubensis*.

In the collection of *P. microdictyi* from San Salvador Island (J.K. 5674), we observed one germinated ascospore that produced antheridia on the apical germ tubes (Fig 2C). It closely resembles germinated ascospores of *Koralionastes angustus* (Kohlmeyer & Volkmann-Kohlmeyer 1987, fig. 12A). This unique feature, *viz.* germ tubes forming antheridia, further supports the close relationship between *Pontogeneia* and *Koralionastes*. However, there are clear differences between them that warrant placement in two separate genera as supported by molecular results. All seven species of *Pontogeneia* are algicolous and have one-layered ascospore walls. Ascospores of four of them are filamentous, the other three are ellipsoidal. All five *Koralionastes* species grow on dead coral rubble among different species of crustose sponges; the ascospores have double walls and are ellipsoidal or fusiform, none is filamentous.

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