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Verrucostoma, a new genus in the Bionectriaceae from the Bonin Islands, Japan

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Abstract: *Verrucostoma freycinetiae* gen. et sp. nov. is described and illustrated from specimens on dead leaves of *Freycinetia boninensis* (Pandanaceae) collected in Hahajima, Bonin (Ogasawara) Islands, Japan. The genus is characterized by pale orange perithecia with protuberances around the perithecial apex, no color change in 3% potassium hydroxide and lactic acid, unitunicate asci, spinulose ascospores and an *Acremonium*-like anamorph. Morphological characters were compared with other genera in the Bionectriaceae and Nectriaceae (Hypocreales). *Verrucostoma* is morphologically similar to *Bionectria* (Bionectriaceae) from which it differs in the formation of conspicuous protuberances around the perithecial apex and the *Acremonium*-like anamorph. Moreover molecular analyses of *Verrucostoma* and other members of the Bionectriaceae and Nectriaceae based on α-actin, large subunit nuclear ribosomal DNA and RNA polymerase II subunit 1 sequences support the conclusions based on morphological data. Our results confirm that *V. freycinetiae* is distinct from other genera among the *Nectria*-like fungi and represents a new genus belonging to the Bionectriaceae.

Key words: Ascomycota, *Freycinetia*, Hypocreales, molecular systematics, Pandanaceae

INTRODUCTION

Seven families, Bionectriaceae, Clavicipitaceae, Cordycipiticeae, Hypocreaceae, Nectriaceae, Niessliaceae and Ophiocordycipitaceae, are currently recognized in the Hypocreales. Fungi in this order usually have brightly colored, soft perithecia with or without a well developed stroma and unitunicate asci (Sung et al. 2007, Kirk et al. 2008). Many hypocrealean species having uniloculate perithecia are included in the Bionectriaceae or Nectriaceae (Rossman et al. 1999). About 600 species of *Nectria*-like fungi, producing uniloculate perithecia with a highly reduced or absent stroma, have been recognized throughout the world and are placed in 32 genera in the Bionectriaceae and 24 genera in the Nectriaceae (Rossman et al. 1999, Schroers et al. 1999, Schoch et al. 2000, Schroers 2001, Samuels et al. 2009). However many described species of *Nectria* (Fr.) Fr. have not been re-evaluated to determine their accurate generic placement. The Bionectriaceae includes *Nectria*-like species that have white to orange or brown perithecia which do not change color in 3% potassium hydroxide (KOH) or 100% lactic acid (LA). Typically members of the Nectriaceae have orange to red perithecial walls that turn dark red or purple in KOH and yellow in LA.

In Jun and Sep 2005 in Hahajima, Bonin (Ogasawara) Islands, Japan, brightly colored perithecial ascomata were discovered on the tips of dead leaves of the endemic species *Freycinetia boninensis* Nakai (Pandanaceae, Toyoda 2003). The Bonins are an archipelago of volcanic islands approximately 1000 km from Tokyo that formed around 48 000 000 y ago (Stem and Bloomer 1992). The result of this isolation has been the evolution of many endemic species, similar to other biogeographically separate islands such as the Galapagos.

The newly discovered fungus is characterized by pale orange perithecia with warty protuberances around the perithecial apex composed of circular to angular, thin-walled cells, an inconspicuous stroma, no color change in 3% KOH and LA, unitunicate asci, spinulose ascospores and an *Acremonium*-like anamorph. The conspicuous perithecial warts that characterize this fungus are formed by localized development of the pseudoparenchymatous cells of

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the perithecial wall. In the present paper a new monotypic genus and a new species are described and discussed based on morphological and molecular data.

MATERIALS AND METHODS

Specimens examined.—They were collected at Hahajima, one of the three main islands of the Bonin archipelago. It is about 1050 km south of Tokyo, 26°80′N, 142°10′E, and features the 463 m Mount Chibusa as its highest point. Fresh specimens of the teleomorph and anamorph were collected from which single ascospores were isolated. All specimens are deposited in the Herbaria of Forest Mycology and Pathology (TFM), Forestry and Forest Products Research Institute, Tsukuba, Ibaraki, Japan, and the U.S. National Fungus Collection (BPI), Beltsville, Maryland.

Fungal isolates.—To obtain cultures from fresh material an ascospore suspension in water taken from crushed perithecia was streaked on 2% water agar (WA) and incubated at 25 C. After 24 h a single germinating ascospore was transferred directly to slants with Difco™ potato dextrose agar (PDA, Detroit, Michigan). A monoascospore isolate is preserved at Genebank, National Institute of Agrobiological Sciences (NIAS), Tsukuba, Ibaraki, Japan.

Morphological observations.—Perithecia were extracted from leaf samples with a needle. A drop of Shear's mounting fluid (Kirk et al. 2008) was applied directly to the perithecia for rehydration. Slides were prepared by hand-sectioning dried material. Morphological structures were examined by light microscopy (Olympus BX50; Olympus, Tokyo, Japan). The color reaction test for the perithecia was carried out with 3% KOH and 100% LA (Rossman et al. 1999). Measurements of continuous characters such as length and width were made with Scion Image software beta 4.0.2 (Scion Corp., Frederick, Maryland). Continuous measurements are based on up to 50 units (numbers given in parentheses) and are reported as the extremes (maximum and minimum in parentheses) separated by the mean plus and minus one standard deviation. The isolate was grown on PDA in 9 cm plastic dishes at 25 C for 7 d in the dark to evaluate growth, colony color and odor. For observation of the *Acremonium*-like anamorph the culture was grown on oatmeal agar (OA; Kirk et al. 2008). OA plates were incubated 2 wk at 25 C in the dark. Top and reverse colony color on PDA was described according to Kornerup and Wanscher (1978).

DNA extraction, PC, and sequencing.—Cultures of the newly described species and representative *Nectria*-like fungi (Bionectriaceae and Nectriaceae) used in the phylogenetic analyses were grown in Difco™ potato dextrose broth in 6 cm diam Petri plates about 3 wk. The mycelial mat was harvested in a laminar flow hood and dried with clean, absorbent paper towels. DNA was extracted with an Ultra Clean™ Plant DNA Isolation Kit (MO BIO Laboratories Inc., Solana Beach, California). Other sequences were obtained from GenBank (TABLE I).

Three partial gene regions were amplified, that is α-actin (*act*), large subunit nuclear ribosomal DNA (LSU) and RNA polymerase subunit one (*rpb1*). The primers used were *act*: T*act*1f (5′-TGGCACCACACCTTCTACAATGA-3′) and T*act*2r (5′-TCTCCTTCTGCATACGGTCGGA-3′) (Samuels et al. 2006); LSU: LRORf (5′-GTACCCGCTGAACTTAAGC-3′) and LR5r (5′-ATCCTGAGGGAAACTTC-3′) (Vilgalys and Hester 1990); and *rpb1*: cRPB1af (5′-CAYCCWGGYT-TYATCAAGAA-3′) and RPB1cr (5′-CCNGCDATNTCRTT RTCCATRTA-3′) (Castlebury et al. 2004). Each 25 µL PCR reaction contained 12.5 µL Promega 2× PCR Master Mix (Promega Corp., Madison, Wisconsin), 1.25 µL each forward and reverse primers (10 mM), 1 µL genomic DNA and sterile distilled water. PCR reactions were placed in an Eppendorf Mastercycler thermocycler (Eppendorf, Westbury, New York) under these conditions: for *act* (1) 2 min at 94 C, (2) 15 cycles of denaturation at 94 C for 30 s, annealing at 65 C for 30 s, and extension at 74 C for 1 min, (3) 30 cycles of denaturation at 94 C for 30 s, annealing at 48 C for 30 s, and extension at 72 C for 1 min, (4) and 10 min at 72 C; for LSU (1) 5 min at 94 C, (2) 35 cycles of denaturation at 94 C for 30 s, annealing at 50 C for 45 s, and extension at 72 C for 1 min, (3) and 7 min at 72 C; and for *rpb1* (1) 5 min at 95 C, (2) 40 cycles of denaturation at 95 C for 1 min, annealing at 50 C for 2 min, and extension at 72 C for 2 min, (3) and 72 C for 10 min. PCR products were cleaned with ExoSAP-IT® (USB Corp., Cleveland, Ohio). Clean PCR products were sequenced at the DNA Sequencing Facility (Center for Agricultural Biotechnology, University of Maryland, College Park, Maryland). Sequences were assembled and edited with Sequencher 4.9 (Gene Codes, Madison, Wisconsin). Sequences were deposited in GenBank (TABLE I), and the alignment file has been submitted to TreeBASE (accession number SN4657).

Phylogenetic analyses.—*act*, LSU and *rpb1* sequences were aligned with MAFFT 6 (Katoh 2008), and the alignment was improved with Mesquite 2.6 (Maddison and Maddison 2009). Maximum likelihood (ML) and Bayesian (BI) analyses were carried out with all sequences, first *act*, LSU and *rpb1* separately, then with the combined datasets. Gaps (insertion/deletions) were treated as missing data. *Aphysiostroma stercorarium* (*Verticillium*-like), *Hypocrea lutea* (*Gliocladium viride*) and *Hypomyces polyporinus* (*Cladobotryum clavisporum*), representative members of the sister family Hypocreaceae, were used as outgroup taxa to determine relationships of the Bionectriaceae and Nectriaceae. ML analyses were performed with RAxML 7.0.4 (Stamatakis et al. 2008). Bootstrap (BP) analyses were done with 1000 replicates. BI was done with MrBayes 3.1.2 (Huelsenbeck et al. 2001, 2002). jModeltest was used to calculate the models of nucleotide substitutions for each gene/partition (Posada 2008). jModeltest estimates the likelihood parameters with PhyML (Guindon and Gascuel 2003). The number of substitution schemes was set to 11, base frequencies +F, rate variation +I and +G, and the base tree for likelihood calculations was set to "ML OPTIMIZED". A total of 88 models were compared. Once the likelihood scores were calculated the models were selected according to the Akaike information criterion (AIC) (Posada and

TABLE I. Isolates and GenBank accession numbers of sequences used in the phylogenetic analyses

Species		Isolate number	GenBank accession number		
Teleomorph	Anamorph		ACT	LSU	RPB1
Aphysiostroma stercorarium Barrasa, A.T. Martínez & G. Moreno	*Verticillium*-like	ATCC 62321	—	AF543792[a]	AY489633[a]
Bionectria byssicola (Berk. & Broome) Schroers & Samuels	*Clonostachys byssicola* Schroers	CBS 123784	GQ505962	GQ506011	GQ506040
Bionectria compactiuscula Schroers	*Clonostachys compactiuscula* (Sacc.) D. Hawksw. & W. Gams	CBS 592.93	GQ505963	GQ506007	GQ506036
Bionectria ochroleuca (Schwein.) Schroers & Samuels	*Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams	CBS 125111	GQ505964	GQ506009	GQ506038
Cosmospora coccinea Rabenh.	"*Verticillium*" *olivaceum* W. Gams	CBS 114050	GQ505967	GQ505990	GQ506020
Cosmospora vilior (Starbäck) Rossman & Samuels	"*Acremonium*" *berkeleyanum* (P. Karst.) W. Gams	G.J.S. 90-217	GQ505965	GQ506010	GQ506039
Cosmospora viliuscula (Samuels, Yoshim. Doi & Rogerson) Rossman & Samuels	"*Acremonium*" cf. *berkeleyanum* (P. Karst.) W. Gams	CBS 455.96	GQ505966	GQ506003	GQ506032
Cosmospora wegeliniana (Rehm) Rossman & Samuels	*Acremonium*-like	G.J.S. 93-15	GQ505968	GQ506006	GQ506035
Cyanonectria cyanostoma (Sacc. & Flageolet) Samuels & Chaverri	*Fusarium* sp.	CBS 101734	GQ505961	FJ474081[a]	GQ506017
Emericellopsis glabra (J.F.H. Beyma) Backus & Orpurt	*Acremonium*-like	A.R. 3614	GQ505969	GQ505993	GQ506023
Hydropisphaera fungicola Rossman, Farr & Newcombe	*Acremonium*-like	CBS 122304	GQ505970	GQ505995	GQ506025
Hydropisphaera peziza (Tode) Dumort.	*Acremonium*-like	CBS 102038	—	AY489730[a]	AY489661[a]
Hypocrea lutea (Tode) Petch	*Gliocladium viride* Matr.	ATCC 208838	—	AF543791[a]	AY489662[a]
Hypomyces polyporinus Peck	*Cladobotryum clavisporum* (D.J. Gray & Morgan-Jones) Rogerson & Samuels	ATCC 76479	—	AF543793[a]	AY489663[a]
Ijuhya paraparilis (Samuels) Rossman & Samuels	*Acremonium*-like	MAFF 241404	GQ505971	GQ506012	GQ506041
Lasionectria mantuana (Sacc.) Cooke	unknown	A.R. 4029	—	GQ505994	GQ506024
Leuconectria clusiae (Samuels & Rogerson) Rossman, Samuels & Lowen	*Gliocephalotrichum bulbilium* J.J. Ellis & Hesselt.	ATCC 22228	—	AY489732[a]	AY489664[a]
Mycoarachis inversa Malloch & Cain	*Acremonium* sp.	ATCC 22107	GQ505972	GQ505991	GQ506021
Nectria austroamericana (Speg.) Rossman	*Gyrostroma austroamericanum* Seeler	A.R. 2808	GQ505960	GQ505988	GQ506016
Nectria balansae Speg.	unknown	CBS 123351	GQ505977	GQ505996	GQ506026
Nectria cinnabarina (Tode : Fr.) Fr.	*Tubercularia vulgaris* Tode	CBS 255.47	GQ505975	GQ505997	GQ506027
Nectria cucurbitula (Tode) Fr.	*Zythiostroma pinastri* (P. Karst.) Höhn.	CBS 259.58	GQ505974	GQ505998	GQ506028
Nectria pseudotrichia (Schwein.) Berk. & M.A. Curtis	*Tubercularia lateritia* (Berk.) Seifert	CBS 451.85	GQ505976	GQ506000	GQ506030
Nectria sinopica (Fr.) Fr.	*Zythiostroma mougeotii* (Fr.) Höhn.	CBS 462.83	GQ505973	GQ506001	GQ506031
Nectriopsis epimycota Samuels	*Acremonium*-like	G.J.S. 95-94	GQ505978	GQ506008	GQ506037

TABLE I. Continued

Species		Isolate number	GenBank accession number		
Teleomorph	Anamorph		ACT	LSU	RPB1
Nectriopsis exigua (Pat.) W. Gams	"*Verticillium*" *rexianum* (Sacc.) Sacc.	G.J.S. 98-32	GQ505979	GQ505986	GQ506014
Neonectria veuillotiana (Sacc. & Roum.) Mantiri & Samuels	*Cylindrocarpon candidulum* (Sacc.) Wollenw.	CBS 125114	GQ505980	GQ506005	GQ506034
Neonectria westlandica (Dingley) Samuels & Brayford	*Cylindrocarpon* sp.	CBS 112464	GQ505959	GQ505987	GQ506015
Ophionectria trichospora (Berk. & Broome) Sacc.	*Antipodium spectabile* Piroz.	CBS 109876	—	AF543790[a]	AY489669[a]
Pseudonectria rousseliana (Mont.) Wollenw.	*Volutella buxi* (Corda) Berk.	CBS 114049	—	U17416[a]	AY489670[a]
Protocreopsis pertusa (Pat.) Samuels & Rossman	*Acremonium*-like	C.T.R. 72-184	GQ505981	GQ506002	—
Roumegueriella rufula (Berk. & Broome) Malloch & Cain	*Gliocladium*-like	CBS 346.85	—	GQ505999	GQ506029
Selinia pulchra (G. Winter) Sacc.	*Acremonium*-like	A.R. 2812	GQ505982	GQ505992	GQ506022
Stilbocrea macrostoma (Berk. & M.A. Curtis) Höhn.	*Stilbella aleuriata* (Berk. & M.A. Curtis) Seifert	G.J.S. 02-125	GQ505983	GQ506004	GQ506033
Verrucostoma freycinetiae	*Acremonium*-like	MAFF 240100	GQ505984	GQ506013	GQ506018
Viridispora alata (Samuels) Samuels & Rossman	*Penicillifer bipapillatus* Samuels	CBS 125123	GQ505985	GQ505989	GQ506019
Viridispora diparietispora (J.H. Mill., Giddens & A.A. Foster) Samuels & Rossman	*Penicillifer furcatus* Polishook	CBS 102797	—	AY489735[a]	AY489668[a]

A.R.: Amy Y. Rossman, USDA-ARS MD USA; ATCC: American Type Culture collection, Manassas, VA, USA; CBS: Centraalbureau voor Schimmelcultures, Utrecht, Netherlands; C.T.R.: Clark T. Rogerson, New York Botanical Garden NY USA; G.J.S.: Gary J. Samuels, USDA-ARS MD USA; MAFF: MAFF Genebank, National Institute of Agrobiological Sciences, Ibaraki, Japan.

[a] Sequences obtained from GenBank.

Buckley 2004) and corrected for smaller samples (AICc option). After jModeltest was run, likelihood settings were: *act*: GTR + I + G (LSET BASE = 0.2041, 0.3497, 0.2361, 0.2101; NST = 6; RMAT = 0.1486, 0.9138, 1.5874, 0.9433, 5.8802, 1.0000; RATES = GAMMA; SHAPE = 1.0960; NCAT = 4; PINVAR = 0.6280); LSU: TIM3 + I + G (LSET BASE = 0.2462, 0.2259, 0.3159, 0.2119) NST = 6; RMAT = 0.4217, 1.7182, 1.0000, 0.4217, 7.6895, 1.0000; RATES = GAMMA; SHAPE = 0.2860; NCAT = 4; PINVAR = 0.5140); and *rpb1*: GTR + I + G (LSET BASE = 0.2096, 0.2983, 0.2957, 0.1964; NST = 6; RMAT = 6.4547, 14.6696, 4.7526, 3.5504, 27.2576, 1.0000; RATES = GAMMA; SHAPE = 0.6920; NCAT = 4; PINVAR = 0.3000). For the BI two independent analyses of two parallel runs and four chains were carried out for 5 000 000 generations with MrBayes. Analyses were initiated from a random tree and trees were sampled every 100th generation. The first 20% of the resulting trees were eliminated as burn-in. A consensus tree (SUMT option) and posterior probabilities (BIPP) were calculated in MrBayes. A reciprocal 70% BP threshold was used to detect topological incongruence among genes/partitions (Mason-Gamer and Kellogg 1996, Reeb et al. 2004).

RESULTS

Phylogenetic analyses.—Sequencing and alignment of the three gene loci for 37 taxa included a total of 1744 characters (base pairs) in the analyses (619 for *act*, 621 for LSU and 504 for *rpb1*) including indels and missing characters. The proportions of phylogenetically informative sites contributing to the combined dataset were 24.2% for *act*, 14.7% for LSU and 55.4% for *rpb1*. Because individual gene tree topologies were not found to be in conflict with a reciprocal 70% BP threshold only a combined phylogeny is presented (FIG. 1). The ML and BI analyses of the combined *act*, LSU and *rpb1* data produced one tree with a log likelihood (Ln) = −6442.23. The Ln for each gene tree were: *act*: −4202.383; LSU: −3131.898; *rpb1*: −8437.834.

As expected the combined ML and BI analyses indicate two major clades: Bionectriaceae (1.00 BIPP, 100% ML bootstrap) and Nectriaceae (1.00 BIPP, 78% ML bootstrap). The fungus investigated in this study is included in the Bionectriaceae and is not contained in any known genera. The closest relatives of the previously unknown fungus are *Protocreopsis*, *Roumegueriella* and *Selinia*. It can be inferred from the phylogenetic analyses that the described species is not closely related to *Bionectria* in the Bionectriaceae or *Viridispora* in the Nectriaceae (Rossman et al. 1999), some species of which have conspicuous perithecial warts similar to those of the fungus described herein. Neither does it appear to be closely related to genera of the Bionectriaceae, *Ijuhya* and *Lasionectria*, characterized by hyphal hairs on the perithecia.

TAXONOMY

Based on our morphological and molecular analyses, the fungus presented here represents a new genus and species described as follows.

Verrucostoma Hirooka, Tak. Kobay. & Chaverri gen. nov.

MycoBank MB 513353

Stromata obscure. Perithecia ad stromate, superficialia, solitaria vel paulo gregaria, latus subglobosa vel doliiformia, alba vel luteola, KOH−, LA−, parietes > 25 µm crassi, apice protuberatiola. Asci unitunicati, clavati, apice simplici. Ascosporae irregulariter distichae, ellipsoideae vel fusiformes, hyalinae.

Typus generic. Verrucostoma freycinetiae Hirooka, Tak. Kobay., & Chaverri

Etymology. Verruco + *-stoma*; "*verruco*" = protuberances, "*stoma*" = perithecial apex.

Stromata basal, thin, inconspicuous. Perithecia superficial, solitary or in small groups, white to pale orange, broadly subglobose to doliiform with protuberances around perithecial apex, not changing color in 3% KOH and LA. Perithecial walls usually more than 25 µm thick, composed of subglobose or angular cells; protuberances around perithecial apex formed of globose or angular, thin-walled cells. Asci unitunicate, narrowly clavate, apex simple. Ascospores ellipsoidal to fusiform, hyaline, 2-celled, spinulose. Anamorph *Acremonium*-like.

Notes. Genus *Verrucostoma* is classified in the Bionectriaceae based on the pale orange, KOH− perithecia and phylogenetic affinities based on molecular data. It is unique in having conspicuous protuberances around the perithecial apex and an *Acremonium*-like anamorph.

Verrucostoma freycinetiae Hirooka, Tak. Kobay. & Chaverri sp. nov. FIGS. 2–18

MycoBank MB 513354

Stromata epiphyllis, obscure. Perithecia ad stromate, superficialia, solitaria vel paulo gregaria, latus subglobosa vel doliiformia, 175–270 µm alta, 180–255 µm diam, alba vel luteola, KOH−, LA−, parietes 25–40 µm crassi, apice protuberatiola. Asci unitunicati, clavate, apice simplici, 42–58 × 7.0–8.5 µm, octospori. Ascosporae irregulariter distichae, ellipsoideae vel fusiformes, 11.3–15.6 × 3.0–4.5 µm, 1-septatae, spinula, hyalinae.

Holotype. JAPAN: TOKYO: Bonin Islands, Ogasawara-mura, Hahajima, Mount Chibusa, on dead leaves of *Freycinetia boninensis* Nakai, 3 Sep 2005, *Tsuyoshi Ono* (BPI 878948–holotype; TFM FPH-7925–isotype)

Etymology. freycinetia + *-e* - indicates the host genus.

Anamorph. Acremonium-like

Mycelium surrounding base of perithecia and on host. Stromata thin, superficial, forming *textura*

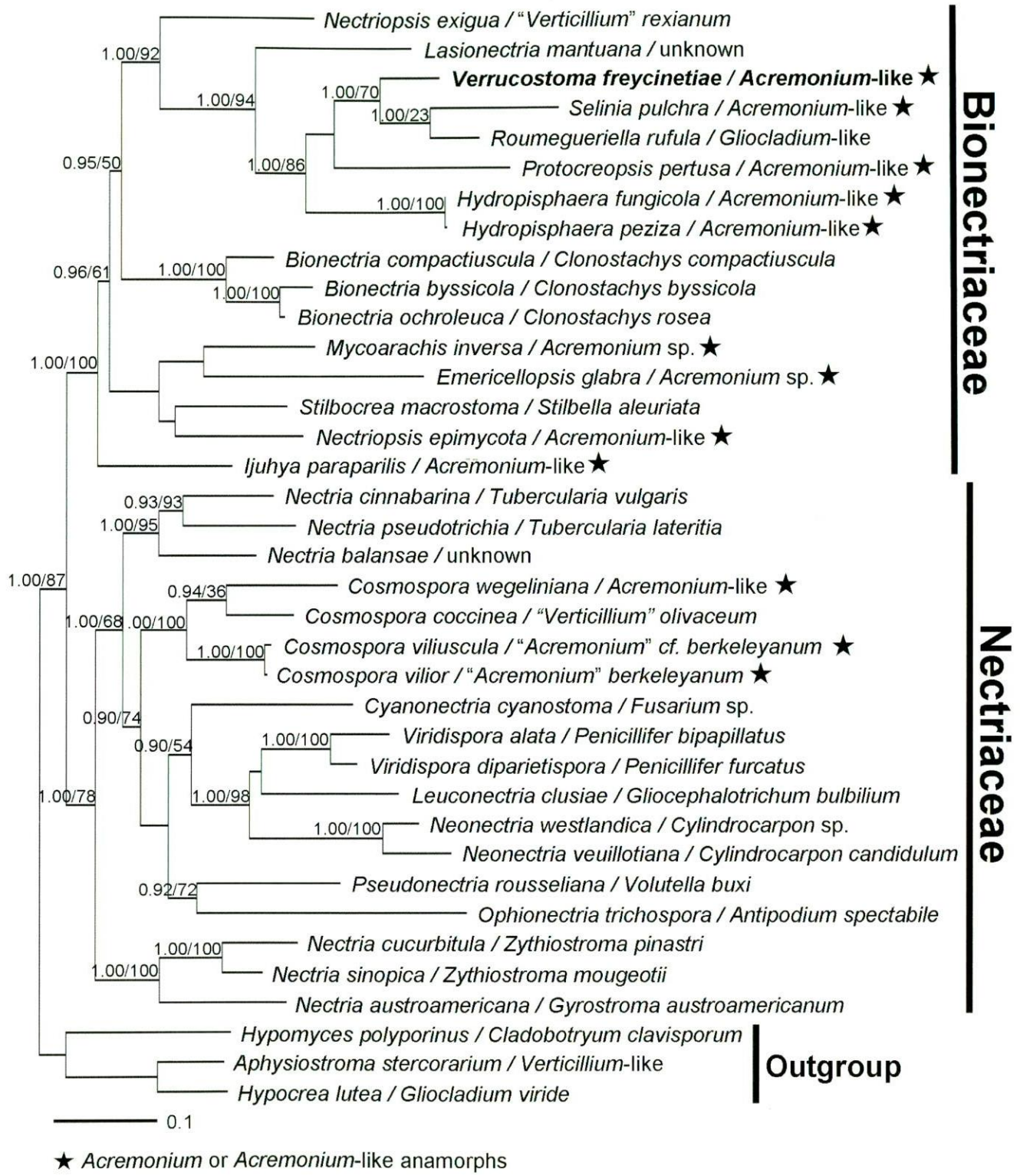
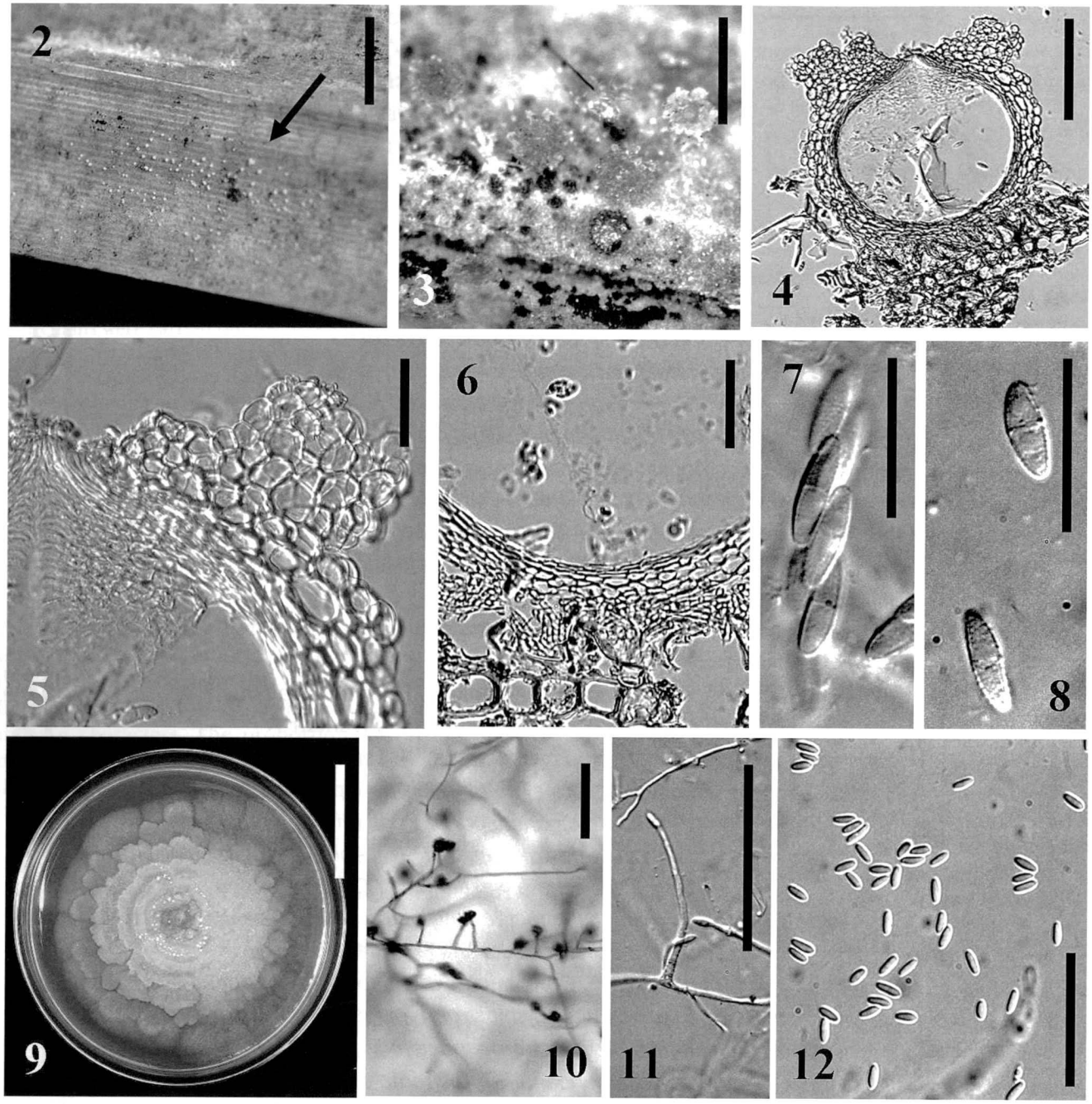
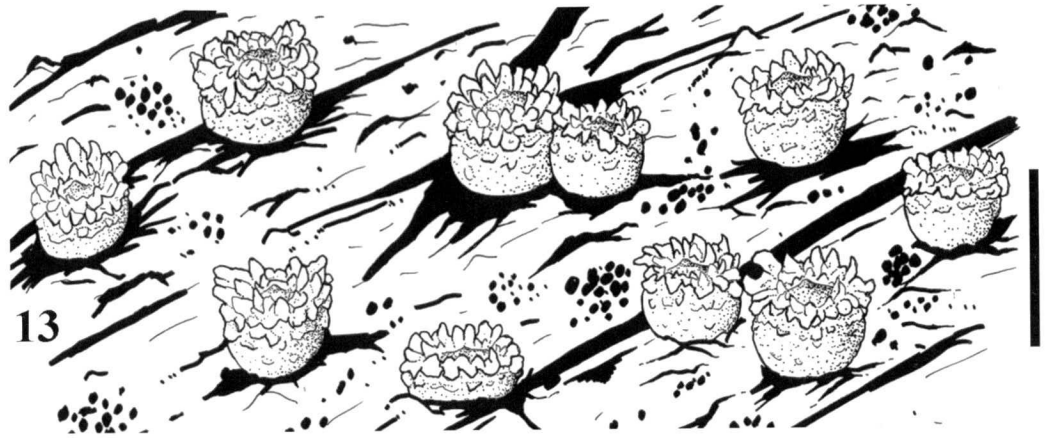


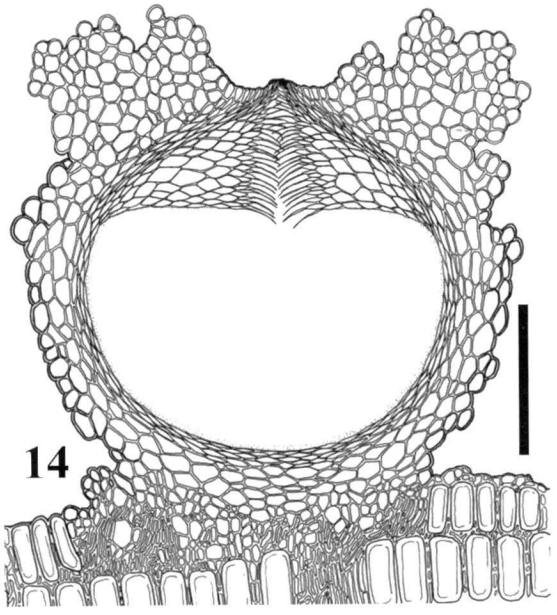
FIG. 1. Bayesian consensus tree (Ln −6442.23) based on combined *act*, LSU and *rpb1* data. BI posterior probabilities/ML bootstrap values indicated at branches.

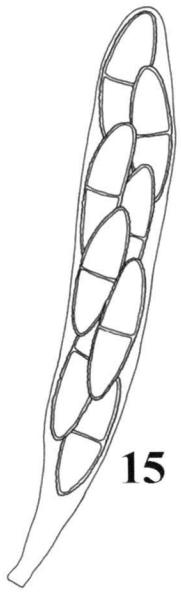


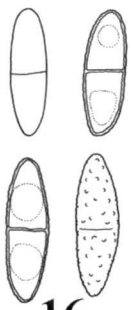
FIGS. 2–12. *Verrucostoma freycinetiae* (Holotype BPI 878948 and isotype TFM FPH-7925). 2. Perithecia on natural substratum (arrow). 3. Close-up of perithecia on natural substratum. 4. Section through the perithecium. 5. Close-up of section through the perithecial apex with protuberance. 6. Close-up of section through the perithecial stroma on leaf of *Freycinetia boninensis*. 7. Ascus. 8. Slightly spinulose ascospores. 9–12. *Acremonium*-like anamorph grown on OA in the dark. (TFM FPH-7926, MAFF 240100). 9. Surface of colony on PDA. 10, 11. Aerial conidiophores on OA. 12. Conidia on OA. Bars: 2, 10 = 2000 μm; 3 = 500 μm; 4 = 100 μm; 6, 11, 12 = 50 μm; 5, 7, 8 = 20 μm; 9 = 3 cm.

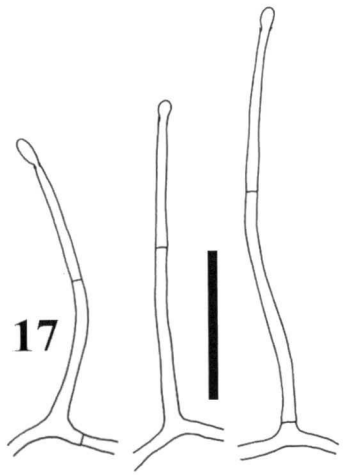
intricata to *t. epidermoidea*. Perithecia solitary or in groups of a few, superficial, erumpent, broadly subglobose to doliiform, with protuberances around perithecial apex, 175–270 μm high × 180–255 μm diam (n = 27), becoming slightly cupulate on drying, white to pale orange, becoming orange when dried, not changing color in KOH and LA, becoming progressively smoother toward base. Perithecial walls ca. 5–6 cells thick, 25–40 μm thick, of two layers; outer layer 15–25 μm thick, cells angular to globose, 10–19 μm diam, with uniformly thickened (0.5–0.8 μm) walls; inner layer 6–12 μm thick, of oblong to

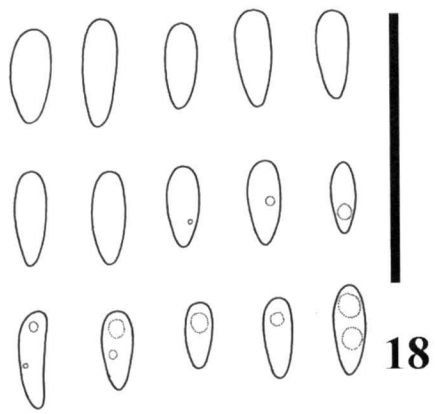












FIGS. 13–18. Schematic figures of *Verrucostoma freycinetiae* (anamorph: *Acremonium*-like) (13–16 Holotype BPI 878948 and isotype TFM FPH-7925; 17, 18, TFM FPH-7926 and MAFF 240100). 13. Perithecia on leaf of *Freycinetia boninensis*. 14. Median section of perithecium. 15. Ascus with eight ascospores arranged biseriately. 16. Spinulose ascospores. 17. Conidiophores on OA. 18. Conidia on OA. Bars: 13 = 500 μm; 14 = 100 μm; 15–18 = 20 μm.

fusiform, thin-walled cells, becoming progressively narrower toward locule. Protuberances forming a white, apical disk, 120–230 µm diam. Each protuberance 25–45 µm high, of globose, thin-walled cells, 8–12 µm diam. Asci unitunicate, narrowly clavate, (42–)45–53(–58) × (7.0–)7.3–8.0(–8.5) µm (n = 30), apex with obscure ring, containing eight spores in two rows. Ascospores ellipsoid to fusiform with ends rounded, equally 2-celled, (11.3–)12.2–14.5(–15.6) × (3.0–)3.4–4.1(–4.5) µm, (n = 50), smooth to finely spinulose, often vacuolate, hyaline.

Colony on OA 17–25 mm diam after 2 wk, lanuginose to wavy, cottony with aerial mycelium, white; reverse white; odor absent. Colony on PDA, wavy, cottony with aerial mycelium, white to pale yellowish-white, aerial mycelium restricted to center or margin; reverse yellowish-white in center and white at margin; odor absent. Sporulation not seen on PDA, on OA abundant, observed within 2–3 wk on aerial mycelium. Conidiophores unbranched, erect, up to 42–62 µm long, 2.5–3.5 µm wide at base, 1–2 µm wide near aperture. Conidiogenous cells long cylindrical, monophialidic, (14.2–)16.8–21.5(–25.5) µm long, (1.2–)1.7–2.0(–2.5) µm wide at base (n = 30), without apical thickening or collarettes. Sporodochia not produced. Conidia aggregated in small slimy heads, hyaline, typically fusiform to clavate, slightly curved, smooth, (5.5–)6.8–7.5(–8.5) × (2.0–)2.2–2.9(–3.2) µm (n = 50). Perithecia not produced in culture.

Distribution. Japan: Tokyo, Bonin Islands, Hahajima, Ogasawara-mura. Known only from type locality.

Habitat. On dead leaves of *Freycinetia boninensis* Nakai.

Specimens examined. JAPAN. TOKYO: Bonin Islands, Ogasawara-mura, Hahajima, Mount Chibusa, on dead leaves of *Freycinetia boninensis*, 3 Sep 2005, *T. Ono* (Anamorph: dried culture TFM FPH-7926, living culture MAFF 240100 ex holotype; holotype BPI 878948, isotype TFM FPH-7925); TOKYO: Bonin Islands, Ogasawara-mura, Hahajima, Mount Chibusa, on dead leaves of *Freycinetia boninensis*, 19 Jun 2005, *Y. Hirooka, Y. Aoki, Y. Fukuda & A. Yumiki* (Paratype BPI 878949).

Notes. Verrucostoma freycinetiae is the only species in the new genus *Verrucostoma* and might be endemic because it is known only on *Freycinetia boninensis*, an endemic plant of the Bonin Islands.

DISCUSSION

Results of the phylogenetic analyses based on three loci support the monophyly of the Bionectriaceae and Nectriaceae and place genus *Verrucostoma* in the Bionectriaceae as suggested by the pale orange, KOH− ascomata (FIG. 1). Molecular analyses, including representatives of 12 genera in the Bionectriaceae, indicate that *Verrucostoma* represents a unique genus within that family. The results also show that the closest relatives of *V. freycinetiae* are *Protocreopsis*, *Roumegueriella* and *Selinia*. *Verrucostoma* differs from *Protocreopsis*, *Roumegueriella* and *Selinia* in many morphological characteristics (TABLE II). Species of *Protocreopsis* produce ascomata immersed in an effused hyphal stroma, have 1-septate ascospores, and occur on monocotyledonous plant debris. *Roumegueriella* produces bright yellow cleistothecia, while ascomata of *Selinia* are immersed in a well developed stroma. Species of the latter two genera produce nonseptate ascospores and generally occur on dung or rotten organic debris. *Verrucostoma freycinetiae* occurs on dead leaves of the monocot *Freycinetia boninensis*, apparently emerging from inside the plant tissue (FIGS. 6, 14) but lacks the effused hyphal stroma characteristic of *Protocreopsis*. Although insect frass was observed on the decaying leaves with the ascomata of *V. freycinetiae*, this new species is unlike the coprophilous *Roumergueriella* and *Selinia* in having 1-septate ascospores.

A separate group of genera in the Bionectriaceae, some of which produce *Acremonium*-like anamorphs, also are characterized by cleistothecial ascomata, including *Emericellopsis* J.F.H. Beyma, *Leucosphaerina* Arx and *Mycoarachis* Malloch & Cain as well as the anamorphic genera *Acremonium* Link, *Geosmithia* Pitt and *Stanjemonium* W. Gams et al. These fungi form a distinct clade within the Bionectriaceae (Rossman et al. 2001, Schroers et al. 2005) and are represented by *Emericellopsis glabra* and *Mycoarachis inversa* in this study (FIG. 1). *Verrucostoma* produces perithecial ascomata on plants and is not closely related to this subgroup of cleistothecial and anamorphic genera within the Bionectriaceae. The anamorphic state of *Verrucostoma* is *Acremonium*-like as is the case in many members of the Hypocreales.

Among the perithecial members of the Bionectriaceae that lack a well developed stroma *Verrucostoma* is distinguished from *Bryonectria* by not occurring on liverworts or mosses and from *Dimerosporiella* and *Nectriopsis* by not occurring on other fungi. *Ijuhya* and *Lasionectria* are distinguished by having a specific type of hyphal hairs on the perithecia that are lacking in *Verrucostoma*, while *Hydropisphaera* and *Ochronectria* have a distinctive ascomatal wall structure that is unlike that of *Verrucostoma* (Rossman et al. 1999). Within the Bionectriaceae, *Verrucostoma* is similar to *Bionectria*, for example *B. byssicola*, *B. lucifer* (Samuels) Schroers & Samuels and *B. subquaternata* (Berk. & Broome) Schroers & Samuels (Schroers 2001), in producing pale orange perithecia with warts or protuberances. These species of *Bionectria* have *Clonostachys* anamorphs. Some species of Nectriaceae have protuberances on the ascomatal wall, such as

TABLE II. Comparison of distinctive morphological features of *Verrucostoma* and morphologically similar related genera in the Hypocreales

	Bionectriaceae							Nectriaceae
Family	*Verrucostoma freycinetii*	*Bionectria* Speg.[a,b]	*Ijuhya* Starbäck[a]	*Lasionectria* (Sacc.) Cooke[a]	*Protocreopsis* Doi[a]	*Roumegueriella* Speg.[a]	*Selinia* P. Karst.[a]	*Viridispora* Samuels & Rossman[a]
Habitat	Herbicolous (*Freycinetia boninensis*)	Herbicolous, corticolous or fungicolous	Herbicolous	Herbicolous or corticolous	Herbicolous (monocotyledonous substrata)	Herbicolous	Dung	Corticolous, sometimes isolated from soil
Position of perithecium	Superficial	Superficial	Superficial	Superficial	Immersed at the base or superficial	Immersed	Immersed	Superficial
Perithecial color	White to pale orange	White, yellow, pale orange, tan or brown	White to pale yellow	Orange to dark red-orange or dark brown	White to orange	Dark yellow to reddish brown	—	Red, orange-brown, tan, or brown
Surface of perithecial apex	Protuberances	Smooth or protuberances	Discoidal to triangular fasciculate hairs	Smooth	Smooth	Smooth (nonostiolate)	Smooth	Smooth
Surface of perithecia	Rough	Smooth, rough or warted	Smooth	With fasciculate hairs	—	—	—	Warted
Ascospore ornamentation	Smooth to spinulose	Striate, smooth to spinulose or slightly warted	Smooth to striate	Smooth	Smooth	Smooth	Typically striate, also smooth, punctuate-striate, or tuberculate	Smooth
Anamorph	*Acremonium*-like *Clonostachys*		*Acremonium*-like	*Acremonium*-like	*Acremonium*-like	*Gliocladium*-like	*Acremonium*-like	*Penicillifer*

[a] Rossman et al. (1999).
[b] Schroers (2001).

Albonectria rigidiuscula Berk. & Broome, *Viridispora alata* and *V. penicilliferi* (Samuels) Samuels & Rossman (Samuels 1989, Rossman et al. 1999). The conspicuous protuberances around the perithecial apex of *V. freycinetiae* are different from those found in the strongly warted species in the Bionectriaceae and Nectriaceae. The structure of the protuberances of *V. freycinetiae* are composed of thin-walled cells that appear irregular in shape. In other species having strongly warted perithecial walls, such as *Albonectria rigidiuscula* and *Bionectria byssicola*, cells of the protuberances are thickened on the outer cell wall appearing capitate or the warts are pyramidal and finite in shape (Rossman et al. 1999, Schroers 2001). Other genera with unique ascomatal wall characteristics include *Ijuhya* and *Lasionectria*, in which the hairs are produced in fascicles (TABLE II), and *Trichonectria*, which produces long solitary hairs (Rossman et al. 1999).

Although the anamorph of *V. freycinetiae* is *Acremonium*-like, it should not be considered a member of *Acremonium s. str.* because it is phylogenetically unrelated to *A. alternatum* Link : Fr, the type species of the genus (Kirk et al. 2008). The present study supports Kirk et al. (2008) in its finding that *Acremonium*, with its simple morphology and ubiquitous distribution, is polyphyletic, having sexual states in most families of the Hypocreales (FIG. 1). In the Bionectriaceae and Nectriaceae, *Acremonium*-like anamorphs are found in at least 20 genera (Rossman et al. 1999, O'Donnell 2000). In the phylogenetic tree (FIG. 1), four species of *Cosmospora* having *Acremonium*-like anamorphs (i.e. *C. coccinea*, *C. vilior*, *C. viliuscula* and *C. wegeliniana*) form a monophyletic group within the Nectriaceae. The type species of *Acremonium*, *A. alternatum*, belongs in the Bionectriaceae, most closely related to the anamorphic genus *Geosmithia*, for which no teleomorph is known, and the cleistothecial genera *Emericellopsis* and *Mycoarachis* (Rossman et al. 2001, Schroers et al. 2005).

Fungi on species in the Pandanaceae have been relatively well studied by McKenzie et al. (2002). A few species of *Nectria* in the broad sense have been described or reported on the host genus *Freycinetia*. Several of these have red to purple ascomata, unlike *Verrucostoma freycinetiae*, and belong in the Nectriaceae, specifically *Gibberella lagerheimii* Rehm, *Neonectria radicicola* (Gerlach & L. Nilsson) Mantiri & Samuels and *N. discophora* (Mont.) Mantiri & Samuels (Saccardo 1899, Mantiri et al. 2001). Three species found on *Freycinetia* belong to the Bionectriaceae but have ascomatal characteristics different from those described in *V. freycinetiae*. *Ijuhya peristomialis* (Berk. & Broome) Rossman & Samuels has distinctive, fasciculate hairs surrounding the perithecial apex (Rossman et al. 1999), *Nectriopsis squamulosa* (Ellis) Samuels has ascomata with spinulose, curved hairs (Samuels 1988), and *Protocreopsis freycinetiae* (Samuels) Samuels & Rossman has ascomata immersed in an effused, hyphal stroma (Rossman et al. 1999).

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