Mycologia, 97(2), 2005, pp. 433–443.

The genus *Podocrella* and its nematode-killing anamorph *Harposporium*

Priscila Chaverri[1]

Department of Plant Pathology, Cornell University, 334 Plant Science Building, Ithaca, New York 14853

Gary J. Samuels

USDA-ARS, Systematic Botany and Mycology Laboratory, Room 304, B-011A, 10300 Baltimore Ave., Beltsville, Maryland 20705

Kathie T. Hodge

Department of Plant Pathology, Cornell University, 334 Plant Science Building, Ithaca, New York 14853

Abstract: Several genera are described in the literature as having morphology similar to the clavicipitaceous genus *Podocrella,* viz. *Atricordyceps, Ophiocordyceps, Wakefieldiomyces* and ''*Cordyceps*'' *peltata*. These genera have capitate-stipitate stromata that gradually expand into a horizontally flattened fertile head that is dark, has strongly protruding perithecia and asci containing eight multiseptate filiform ascospores. These ascospores disarticulate at the middle septum to form two lanceolate multiseptate part-ascospores. In this study several specimens of the above-mentioned genera, including the types, were examined to determine whether they are congeneric with *Podocrella*. This study also reveals the connection of *Podocrella* to its anamorph genus, *Harposporium,* and its relationship to several other clavicipitaceous genera, based on cultural data and large subunit nuclear ribosomal DNA (LSU) sequences. Nematode predation of the *Harposporium* anamorph of *P. peltata* is demonstrated. The results show *Podocrella* and selected *Harposporium* LSU sequences form a monophyletic group and that this clade is closely related to *Aschersonia*. A new species of *Podocrella* from Costa Rica, *P. fusca,* is described, new combinations made for *P. peltata* and *P. harposporifera,* and a key to the known species is presented.

Key words: Anamorph-teleomorph connection, Ascomycota, Clavicipitaceae, *Hirsutella,* Hypocreales, large subunit nuclear ribosomal DNA, molecular phylogenetics, synanamorphs, systematics

Accepted for publication 7 Oct 2004.

[1] Corresponding author. Current address: USDA-ARS, Systematic Botany and Mycology Laboratory, Room 304, B-011A, 10300 Baltimore Ave., Beltsville, Maryland 20705. Email: pc234@cornell.edu

INTRODUCTION

The genus *Podocrella* Seaver was erected based on the single species *P. poronioides* Seaver (1928). Although it was described originally from wood in Trinidad, its typically clavicipitacean asci and stroma led Rossman et al (1999) to suspect that the true host might be an arthropod overlooked by the collector; it has not been reported since its original description. *Wakefieldiomyces* Kobayasi (1981) and *Atricordyceps* Samuels (1983) resemble *Podocrella* in their dark, peltate stromata and other features, and we suggest they should be considered synonyms of *Podocrella*. *Wakefieldiomyces* is based on *Cordyceps peltata* E.M. Wakefield (Wakefield and Groves 1916), which was described from St Vincent, an island in the Lesser Antilles; monotypic *Atricordyceps* was described from New Zealand. Petch (1931) included *C. peltata* in the genus *Ophiocordyceps* Petch, along with three other species. *Ophiocordyceps blattae,* the type of the genus, is morphologically distinct from *Podocrella,* therefore we do not consider *Ophiocordyceps* to be congeneric with *Podocrella*.

Of the several species that we can consider for inclusion in *Podocrella,* an anamorph is known for only one, *Atricordyceps harposporifera* Samuels, which was reported to have an anamorph attributable to *H. anguillulae* Lohde, the type of the genus *Harposporium* (Samuels 1983). Molecular data reported by Sung et al (2001) confirmed the relationship of *A. harposporifera* and another *Harposporium* species, *H. helicoides* Drechsler. The *Harposporium* anamorph of *Cordyceps peltata* is described here based on a collection from Costa Rica.

Harposporium species are fungi common in soil; many have been found infecting various nematodes, rotifers or tardigrades, including *Prismatolaimus* spp. and *Rhabditis* spp., among others (Barron 1977, Drechsler 1968, Viaene 1996). Although *Harposporium* species generally are not known to infect insects, their possible teleomorph species have been reported only from arthropods. *Atricordyceps harposporifera* was isolated from a centipede, and *Cordyceps peltata* was found on infected larvae of *Cryptorhynchus* sp. (Coleoptera). The host of the type specimen of *Podocrella poronioides* was not apparent, but Rossman et al (1999) hypothesized that it possibly developed from an insect larva buried in the wood. *Harpospor-*

ium janus Shimazu & Glockling, which has the ability to infect beetle larvae and nematodes, exhibited a gradual change from a synnematous form on the beetle larva to a typical *Harposporium* form on the nematode (Shimazu and Glockling 1997).

In this study we evaluate the synonymy of several *Cordyceps*-like genera with dark, peltate stromata and *Harposporium* anamorphs, use partial LSU sequences to investigate their relationships with other clavicipitaceous fungi including selected *Harposporium* spp. and describe a new species in this group from Costa Rica. A key to known species of *Podocrella* is presented. Keys to described *Harposporium* species were published by Esser and El-Gholl (1992) and Gams and Zare (2003).

MATERIALS AND METHODS

Morphological examination.—Type specimens of *Cordyceps peltata, Atricordyceps harposporifera* and *Podocrella poronioides* were obtained respectively from the Herbarium of the Royal Botanic Gardens, Kew (K), the New Zealand Fungal Herbarium (PDD) and the William and Lynda Steere Herbarium (NY). The two specimens from Costa Rica were obtained from the U.S. National Fungus Collection (BPI) (*C. peltata,* specimen BPI 749196) and the Botany Department Herbarium of the National Biodiversity Institute of Costa Rica (INB) (*Podocrella* sp., specimen INB 3835871). Cultures were obtained from Agricultural Research Service Collections of Entomopathogenic Fungi, U.S. Plant, Soil and Nutrition Lab, Ithaca, New York (ARSEF).

Herbarium specimens were rehydrated briefly in distilled water with a trace of Tween® 80 (J.T. Baker Chemical Co., Phillipsburg, New Jersey). Rehydrated stromata were supported by Tissue-Tek O.C.T. Compound 4583 (Miles Inc., Elkhart, Indiana) and sectioned at a thickness ca. 15 µm with a freezing microtome to observe and measure the characteristics of the stroma tissue and perithecia. Asci and ascospores also were characterized. Color terminology is from Kornerup and Wanscher (1978).

A culture from *C. peltata* BPI 749196 (culture G.J.S. 96-242 = ARSEF 5410) was obtained from single-ascospore isolations made with a micromanipulator. The ascospores were germinated and grown on CMD (Difco cornmeal agar [Difco Laboratories, Detroit, Michigan] + 2% dextrose + 1% antibiotic solution (0.2% Sigma Streptomycin Sulfate [Sigma-Aldrich Corp., St Louis, Missouri] + 0.2% Sigma Neomycin Sulfate + distilled water). Morphological observations of the anamorph were based on cultures grown on Difco malt-extract agar (MEA) for 2 wk at 22 C. To study the germination of the various conidial types, conidia were harvested from a mature colony by washing it with sterile distilled water containing a trace of Tween 80. A drop of the suspension was spread on 1.8% water agar on glass slides and observed under the compound microscope at 400× for 24 h.

Measurements of continuous characters such as length were made with the beta 4.0.2 version of Scion Image software (Scion Corp., Frederick, Maryland). Confidence intervals ($\alpha = 0.05$), minimum and maximum values for 10–30 anamorph and teleomorph measurements (except where indicated) were calculated with Systat 8.0 (SPSS Inc., Chicago, Illinois).

Nematode bioassay.—The ability of the *C. peltata* isolate ARSEF 5410 to infect and kill nematodes was assessed in vitro using the method described in Hodge et al (1997). A few drops of a culture of an unidentified rhabditid nematode isolated from New York turf samples were applied to a 2 mo old culture of ARSEF 5410 on MEA. The dishes were incubated at room temperature and observed 10 d for infection. Infection was confirmed by picking nematodes with a fine needle, mounting them in lactic acid-cotton blue and observing them with a compound microscope.

DNA extraction, PCR and sequencing.—Cultures of *H. anguillulae* (ARSEF 5407 and 5593), *H. cycloides* Drechsler (ARSEF 5599) and *C. peltata* (ARSEF 5410) were grown on Difco potato-dextrose agar ca. 2 wk. Mycelium was harvested in a laminar flow hood by scraping, then suspended in extraction buffer and frozen. Extraction of genomic DNA and PCR amplification was done using methods similar to those described by Sung et al (1999). The large subunit nuclear ribosomal DNA (LSU) primers used were LR0R (5′-ACCCGCTGAACTTAAGC-3′) and LR5 (5′-TCCTGAGGGAAACTTCG-3′), which produced a sequence of ca. 800 nucleotides (Vilgalys and Hester 1990). The resulting products were purified with the QIAquick™ PCR Purification Kit (Qiagen Inc., Valencia, California). Sequencing of forward and reverse strands was performed at the Bio-Resource Center, Cornell University, Ithaca, New York. Sequences were edited and assembled with Sequencher 4.1 (Gene Codes, Madison, Wisconsin) and SeqEd (Applied Biosystems, Branchburg, New Jersey). Sequences have been deposited in GenBank (TABLE I) and the alignment in TreeBase (study number S 1115, http://treebase.bio.buffalo.edu/treebase/).

Phylogenetic analysis.—*Harposporium anguillulae* (ARSEF 5407 and 5593), *H. cycloides* (ARSEF 5599) and *C. peltata* (ARSEF 5410) LSU sequences were aligned to several sequences downloaded from GenBank (TABLE I). Clustal X 1.81 (Thompson et al 1997) was used to align the sequences, and alignment was refined by hand. Maximum parsimony (MP), neighbor-joining (NJ), and Bayesian analyses were carried out with all sequences. MP analysis was done in PAUP* version b8 (Swofford 1999) using a heuristic search, with a starting tree obtained via 1000 random stepwise addition sequences, tree-bisection-reconnection as the branch-swapping algorithm, and MulTrees off. Bootstrap values from 1000 replicates were calculated with a ''fast'' stepwise addition search. NJ trees were constructed with the Kimura-2-parameter model in PAUP* and bootstrapping was replicated 500×. MrBayes (Huelsenbeck 2000) was used to reconstruct phylogenetic trees with the Bayesian approach (Mau et al 1999, Rannala and Yang 1996). The nucleotide substitution model was calculated with Modeltest 3.0 (Posada and Crandall 1998). The model selected was GTR, with variable base frequencies (freqA = 0.2285, freqC

TABLE I. Sequences used for phylogenetic analyses

Species	Geographic origin[a]	Culture/Specimen number	GenBank accession number and source of LSU sequence
Harposporium anguillulae	U.S.A.	ARSEF 5407	AY 636080
Harposporium anguillulae	Japan	ARSEF 5593	AY 636081
Harposporium cycloides	Japan	ARSEF 5599	AY 636083
Harposporium helicoides	Canada	ARSEF 5354	AF 339527[b]
Podocrella (=Cordyceps) peltata	Costa Rica	ARSEF 5410, BPI 749196	AY 636082
Podocrella (=Atricordyceps) harposporifera	New Zealand; type	ARSEF 5472, PDD 42208	AF 339519[b]
Aschersonia aleyrodis		CR 01	AY 636079, M. Liu
Balansia obtecta			U 17395[b]
Balansia strangulans			U 57679[b]
Berkelella stilbigera			AY 259545[b]
Chaunopycnis alba			AF 245296[b]
Cordycepioideus bisporus			AF 009654[b]
Cordyceps capitata			U 57086[b]
Cordyceps capitata			AB 027364[b]
Cordyceps coccidiicola			AB 031196[b]
Cordyceps inegoensis			AB 027368[b]
Cordyceps japonica			AB 027366[b]
Cordyceps jezoensis			AB 027365[b]
Cordyceps kansashiana			AB 027371[b]
Cordyceps militaris			AY 184966[b]
Cordyceps ophioglossoides			U 47827[b]
Cordyceps sinensis			AB 067712[b]
Cordyceps subsessilis			AF 373285[b]
Epichlöe amarillans			U 57680[b]
Hyperdermium bertonii			AF 242354[b]
Hypomyces broomeanus			AF 160231[b]
Hypomyces orthosporus			AF 160241[b]
Paecilomyces tenuipes			AB 027380[b]
Polycephalomyces formosus			AY 259544[b]
Tolypocladium cylindrosporum			AF 245301[b]
Tolypocladium inflatum			AF 373286[b]
Verticillium balanoides			AF 339540[b]
Verticillium campanulatum			AF 339543[b]

[a] Geographic origin is indicated only for the sequences relevant to this study.
[b] Sequences obtained from GenBank.

= 0.2601, freqG = 0.3250, freqT = 0.1864), substitution model R(a) [A–C] = 0.8060, R(b) [A–G] = 3.6576, R(c) [A–T] = 0.5209, R(d) [C–G] = 1.5145, R(e) [C–T] = 9.1042, R(f) [G–T] = 1.000, proportion of invariable sites = 0.6507, and a gamma distribution shape parameter = 0.5662. MrBayes was run for 500 000 generations. A consensus tree was calculated with the 50% majority rule option in PAUP*. Outgroup species were *Hypomyces broomeanus* L.R. & C. Tulasne (in GenBank as *Sphaerostilbella broomeana*) and *Hypomyces orthosporus* K. Põldmaa.

RESULTS

Morphological analyses.—All specimens examined have capitate-stipitate stromata with a flat cap and a tuberculate upper surface; *C. peltata* specimens have the least tuberculate surface. The type specimens of *A. harposporifera* and *P. poronioides* have dark green almost black stromata, whereas specimens of *C. peltata* and the new species of *Podocrella* from Costa Rica have dark brown stromata. The ascospores of all the specimens are multiseptate and disarticulate at the middle, forming two lanceolate multiseptate part-ascospores. The specimen of *Podocrella* sp. from Costa Rica (INB 3835871) has dark brown stromata and larger part-ascospores 57–62 μm long; it is morphologically distinct from the known *Podocrella*-like species.

The two anamorphs that originated from the *Podocrella*-like specimens, namely *C. peltata* (ARSEF 5410) and *A. harposporifera* (Samuels 1983), are typical of *Harposporium*. The conidiophores are erect, unbranched and bear phialides in pairs or whorls.

The phialides are subglobose with a cylindrical neck and the conidia are arcuate, smooth and hyaline. The *C. peltata* culture ARSEF 5410 produced two other synanamorphs, a *Hirsutella*-like synanamorph, with awl-shaped phialides and fusiform conidia, and an arthroconidial anamorph.

Phylogenetic analyses.—Eight hundred base pairs of 33 partial LSU sequences were included in the analysis. The aligned matrix included 645 constant and 155 polymorphic characters, of which 115 were informative. Distance (NJ) and character-based (MP and Bayesian) analyses yielded identical topologies. A Bayesian tree with the best log-likelihood is presented (FIG. 1).

Atricordyceps harposporifera, Harposporium anguillulae, H. cycloides, H. helicoides and *C. peltata* form a monophyletic group supported by a posterior probability of 94% in the Bayesian analysis and a bootstrap value of 70% in the NJ tree (FIG. 1). Although this group does not receive bootstrap support in the MP analysis, the consensus (50% majority rule) of three most parsimonious trees supports this clade by 100% probability. Within the *Podocrella/Harposporium* clade there is a subclade comprising *Atricordyceps harposporifera, C. peltata* and *H. cycloides,* supported by 94% posterior probability, and a separate subclade comprising *H. helicoides* and two isolates of *H. anguillulae,* supported by 80% posterior probability. LSU phylogenetic analyses groups *Podocrella/Harposporium* as sister of *Aschersonia aleyrodis* (teleomorph *Hypocrella libera* Sydow) (FIG. 1).

Conidial germination and nematode bioassay.—We demonstrated that the *C. peltata* culture ARSEF 5410 could infect and kill rhabditid nematodes in vitro. The *Harposporium* anamorph of *A. harposporifera* (ARSEF 5472, culture ex type) shares this ability (Hodge unpubl). All three different types of conidia of *C. peltata* ARSEF 5410 had germinated after 22 h. *Harposporium* and *Hirsutella*-like conidia formed a single germ tube that arose near the center of the conidium (FIG. 28). Arthroconidia typically formed subglobose *Harposporium*-like conidiogenous cells upon germination, which in turn produced typical conidia, or very rarely produced several lateral germ tubes just below one end of the arthroconidium (FIGS. 24, 25). Whereas most of the *Harposporium* and *Hirsutella*-like conidia germinated, only about 50% of arthroconidia had germinated after 30 h, when observation was discontinued.

The *Harposporium* conidia of ARSEF 5410 were observed in the esophagus of rhabditid nematodes within 2 h of exposure, indicating that the nematodes were ingesting the conidia (FIG. 29). Many dead nematodes were observed on Days 2 and 3. On the fourth day, numerous nematode cadavers were observed bearing erect conidiophores and the arcuate conidia typical of *Harposporium* (FIGS. 30, 31). Arthroconidia and *Hirsutella*-like conidia were not observed to be associated with infection, but due to the difficulty of following the course of infection we cannot rule out their involvement.

DISCUSSION

Phylogenetic analyses of LSU sequences revealed that the available sequences of *Podocrella*-like species and selected *Harposporium* species, including the type, *H. anguillulae,* form a monophyletic group supported by high posterior probability and bootstrap values (FIG. 1). Morphology of all the specimens examined was consistent with the defining features of the type of the genus, *P. poronioides,* thus we deem them congeneric. We conclude that *Atricordyceps, Cordyceps peltata* (≡ *Wakefieldiomyces peltatus* ≡ *Ophiocordyceps peltata*), *Podocrella poronioides* and *Podocrella* sp. (INB 3835871) are congeneric, and the anamorphs of *Podocrella,* where known, are *Harposporium* species. New combinations are presented in the taxonomy section. The *Podocrella* specimen collected in Costa Rica (INB 3835871) is morphologically distinct from other known species of *Podocrella*. We describe it as a new species, *P. fusca*. A key to the four known species of *Podocrella* is provided.

Podocrella is distinguished from other genera in the Clavicipitaceae by its shallow ascus cap and thick multiseptate ascospores that disarticulate at the middle septum. The species that Petch (1931) placed in *Ophiocordyceps, O. blattae* Petch, *O. unilateralis* (L.R. & C. Tulasne) Petch, and *O. rhizoidea* (v. Höhnel) Petch, have clavate stromata, filiform multiseptate ascospores that do not disarticulate at the middle septum; these species do not resemble any of the *Podocrella* species treated here. Based on LSU sequences, *Podocrella/Harposporium* species are closely related to *Aschersonia aleyrodis* (FIG. 1). The relationship of *Podocrella/Harposporium* and *Aschersonia* to other genera in the Clavicipitaceae is not well resolved based on LSU gene genealogy and the limited taxa sampled in this study.

In addition to having typical *Harposporium* conidiophores and conidia, the anamorph of *P. peltata* (≡ *Cordyceps peltata*) has *Hirsutella*-like and arthroconidial synanamorphs. *Hirsutella*-like synanamorphs have been observed in *H. anguillulae, H. cerberi* W. Gams et al, *H. drechsleri* Barron and *H. janus* Shimazu & Glockling (Barron 1972, Hodge et al 1997, Shimazu and Glockling 1997), but these differ from the synanamorph of *P. peltata*. Arthroconidia have been reported for *H. arthrosporum* Barron, *H. cerberi,*

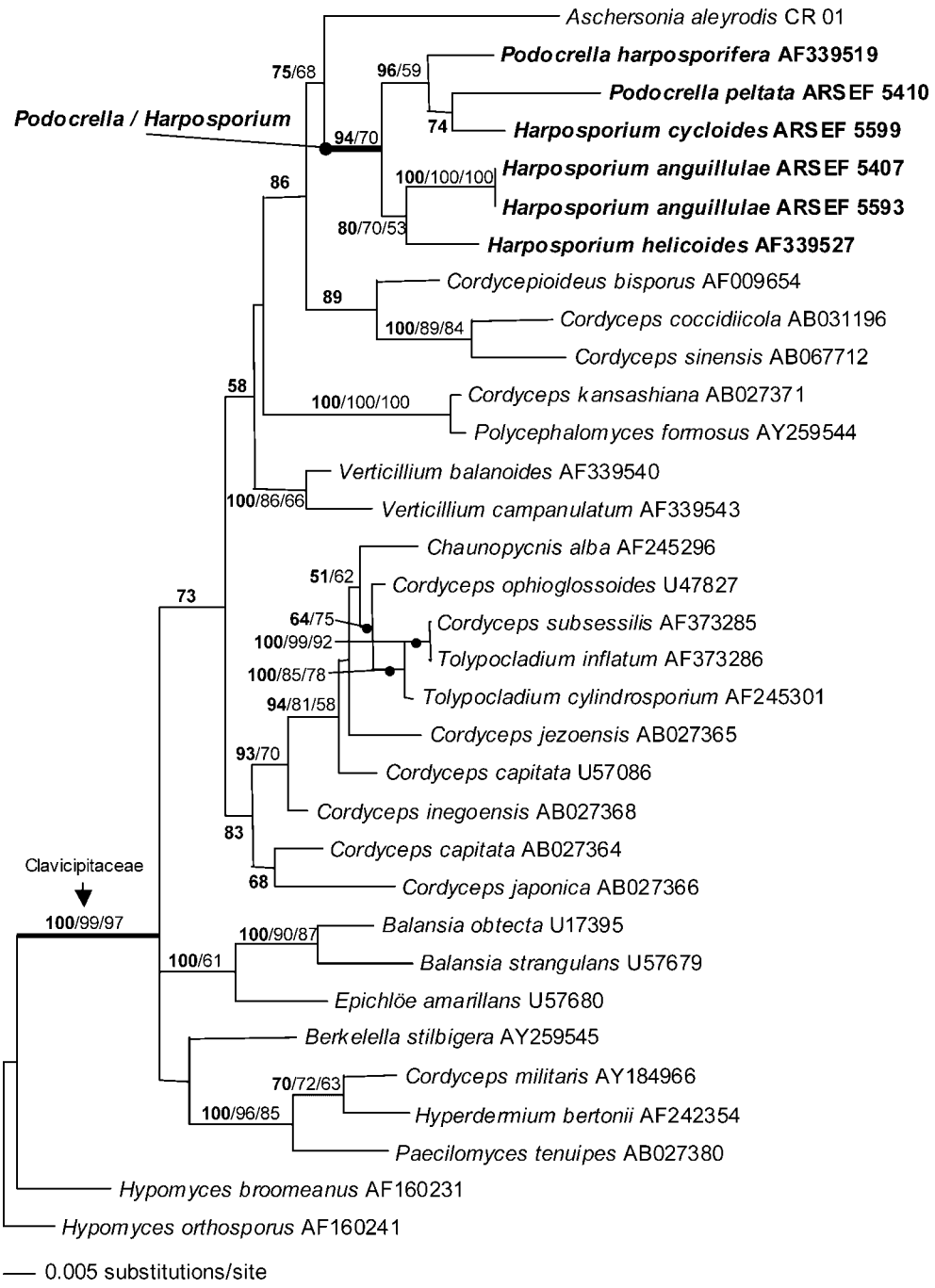
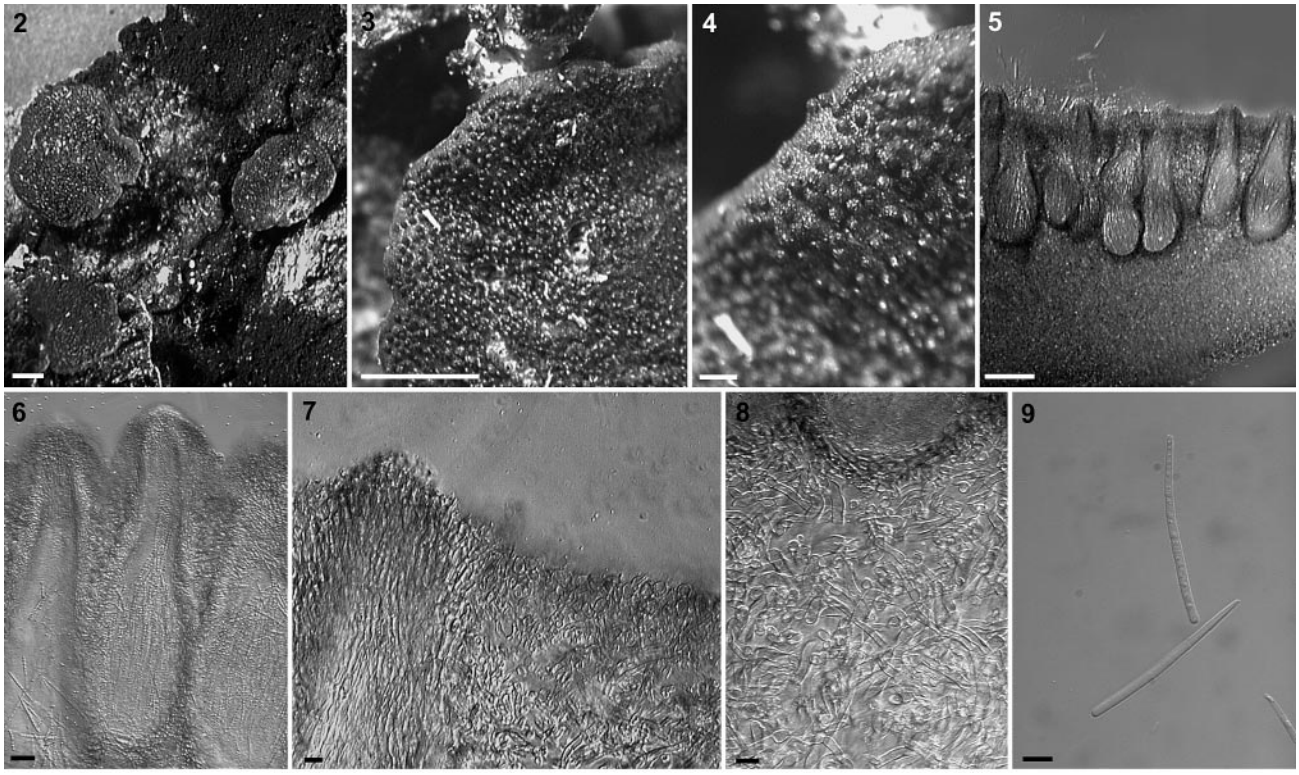


FIG. 1. LSU cladogram with the best log-likelihood (−3320.83) obtained in the Bayesian analysis. Numbers at nodes indicate values for posterior probability (%) in Bayesian analysis (bold)/neighbor-joining bootstrap/maximum parsimony bootstrap. Maximum parsimony (MP) analysis scores: 645 characters are constant, 40 characters are parsimony uninformative, 115 characters are parsimony informative (14.4%), 3 MP trees, 419 steps, CI: 0.47, RI: 0.62, HI: 0.53.

and *H. diceraeum* Drechsler (Aoki and Saikawa 1992, Barron 1979, Hodge et al 1997). Our data suggest that isolates that have arcuate conidia have been attributed to *Harposporium anguillulae* and they may reflect several cryptic species. Although two isolates of *H. anguillulae* (ARSEF 5407 and ARSEF 5593) had identical LSU sequences, the two *Podocrella* anamorphs (ARSEF 5472 and ARSEF 5410) are genetically different. *Harposporium lilliputanum* Dixon and a *H. anguillulae*-like isolate described by Hodge et al (1997) also appear to be members of this complex. We have not named the anamorphs of the *Podocrella* species studied because the genus *Harposporium* needs critical revision and the main objectives of this



FIGS. 2–9. *Podocrella fusca* (HOLOTYPE). 2–4. Stromata. 5–8. Longitudinal section of stroma. 5, 6. Perithecia. 7. Tissue of surface of stroma. 8. Tissue below perithecia. 9. Part-ascospores. Bars: 2 = 1 mm; 3 = 0.5 mm; 4, 5 = ca. 200 μm; 6 = 50 μm; 7–9 = 10 μm.

paper were to deal with *Podocrella* and link it to its anamorph genus. Keys to *Harposporium* species have been presented by Esser and El-Gholl (1992) and Gams and Zare (2003).

TAXONOMY

Podocrella Seaver, Mycologia 20:57. 1928.

Anamorph: *Harposporium* Lohde, where known.

Stromata dark green or dark brown, almost black, stipitate, expanding into a horizontally flattened fruiting cap, upper surface of cap slightly to strongly tuberculate due to protruding perithecia; on substrata only cap visible because stipe typically buried in the wood. Most superficial layer of stroma formed of globose to angular thin-walled cells; inner tissue of stroma of textura intricata. Perithecia ovoid to obpyriform, 460–850 × 160–300 μm. Asci cylindrical, somewhat capitate, with shallow apical caps. Ascospores hyaline, smooth, multiseptate, filiform to fusiform, disarticulating at the middle septum forming two lanceolate multiseptate part-ascospores; part-ascospores 37.0–70.0 × 1.5–3.0 μm.

Type species. *Podocrella poronioides* Seaver.

1. Podocrella fusca Chaverri et K.T. Hodge, sp. nov. FIGS. 2–9

Anamorph: Unknown.

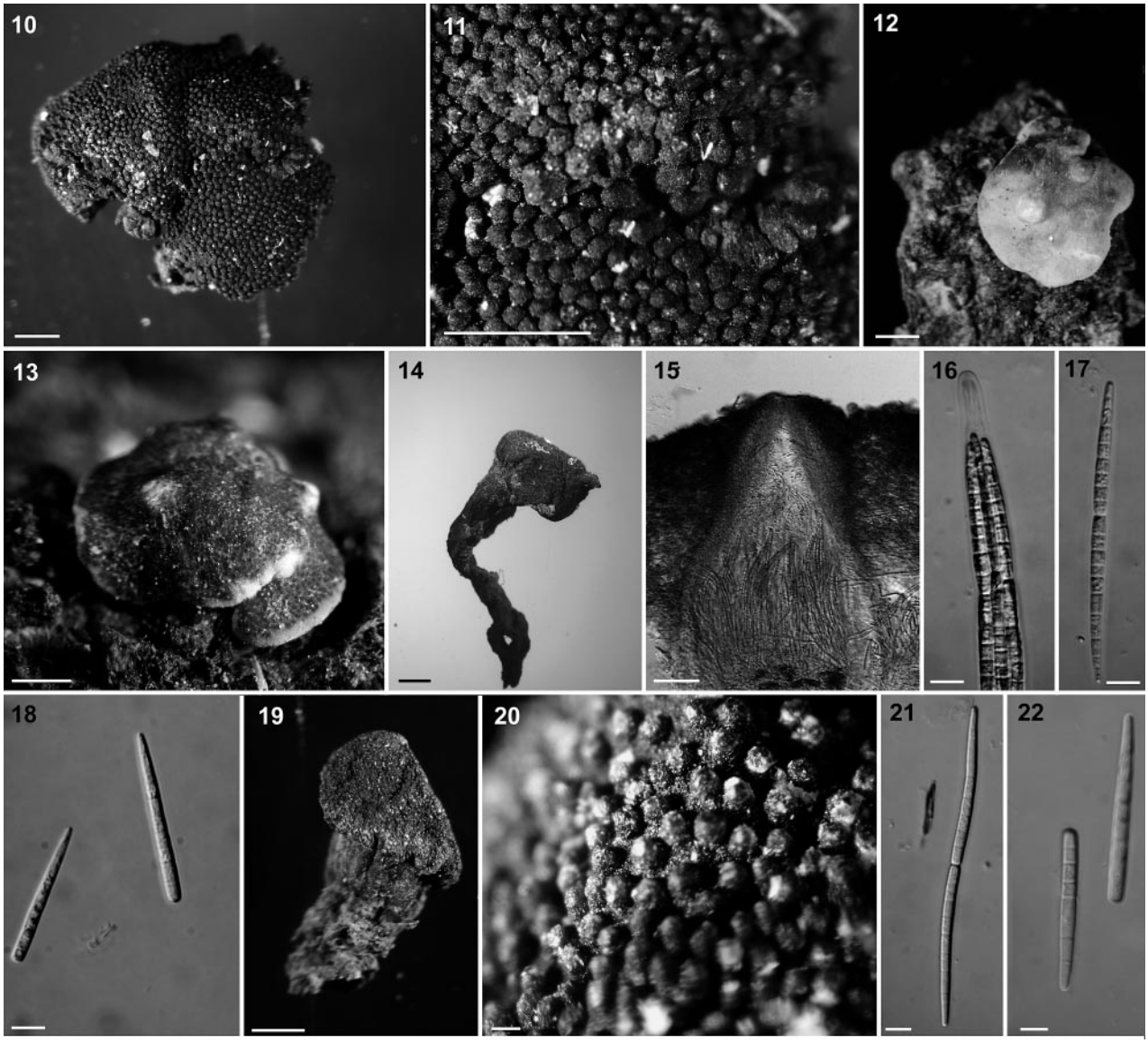
Stromata stipitata-capitata, fusca, caput tuberculata superficiei, 3.0–4.5 diam. Ascosporae multicellulares, ad medioseptum disarticulatae, incolora, partis lanceolata, (43.0–)56.7–62.2(–75.5) × (1.7–)2.7–3.0(–3.5) μm. Holotypus INB 3835871.

Stromata solitary, dark brown, stipitate-capitate, upper surface of cap strongly tuberculate due to protruding perithecia, cap circular in outline, 3.0–4.5 mm diam (n = 5). Stroma outer *tissue textura* angularis, cells thin-walled, (4.5–)6.0–7.0(–9.2) μm diam. Stroma inner tissue *textura intricata,* hyphae thin-walled, (3.2–)4.2–5.0(–6.0) μm diam. Perithecia obpyriform, (465–)520–563(–647) × (161–)190–209(–241) μm. Ascospores multiseptate, hyaline, smooth, disarticulating at middle septum; part-ascospores lanceolate, multiseptate, (43.0–)56.7–62.2(–75.5) × (1.7–)2.7–3.0(–3.5) μm.

Habitat. On remnants of an arthropod buried in decaying wood.

Known distribution. Costa Rica (type locality).

Holotype. COSTA RICA. LIMÓN: Valle de la Estrella, Biological Reserve Hitoy Cerere, 100 m eleva-



FIGS. 10–22. FIGS. 10, 11. Stroma of *Podocrella harposporifera*. FIGS. 12–18. *Podocrella peltata*. 12. Immature stroma. 13, 14. Mature stroma. 15. Longitudinal section of perithecium. 16. Ascus and ascus cap. 17. Ascospore. 18. Part-ascospores. FIGS. 19–22. *Podocrella poronioides*. 19, 20. Stroma. 21. Ascospore. 22. Part-ascospores. FIGS. 10–13, 16–22: Holotypes; 14, 15: BPI 749196. Bars: 10–14, 19 = 1 mm; 15, 20 = ca. 50 μm; 16–18, 21 = 10 μm; 22 = 5 μm.

tion, possibly on remnants of arthropod buried in wood, 7 Jul 1999, *P. Chaverri (53), G.J. Samuels, L. Umaña* (INB 3835871).

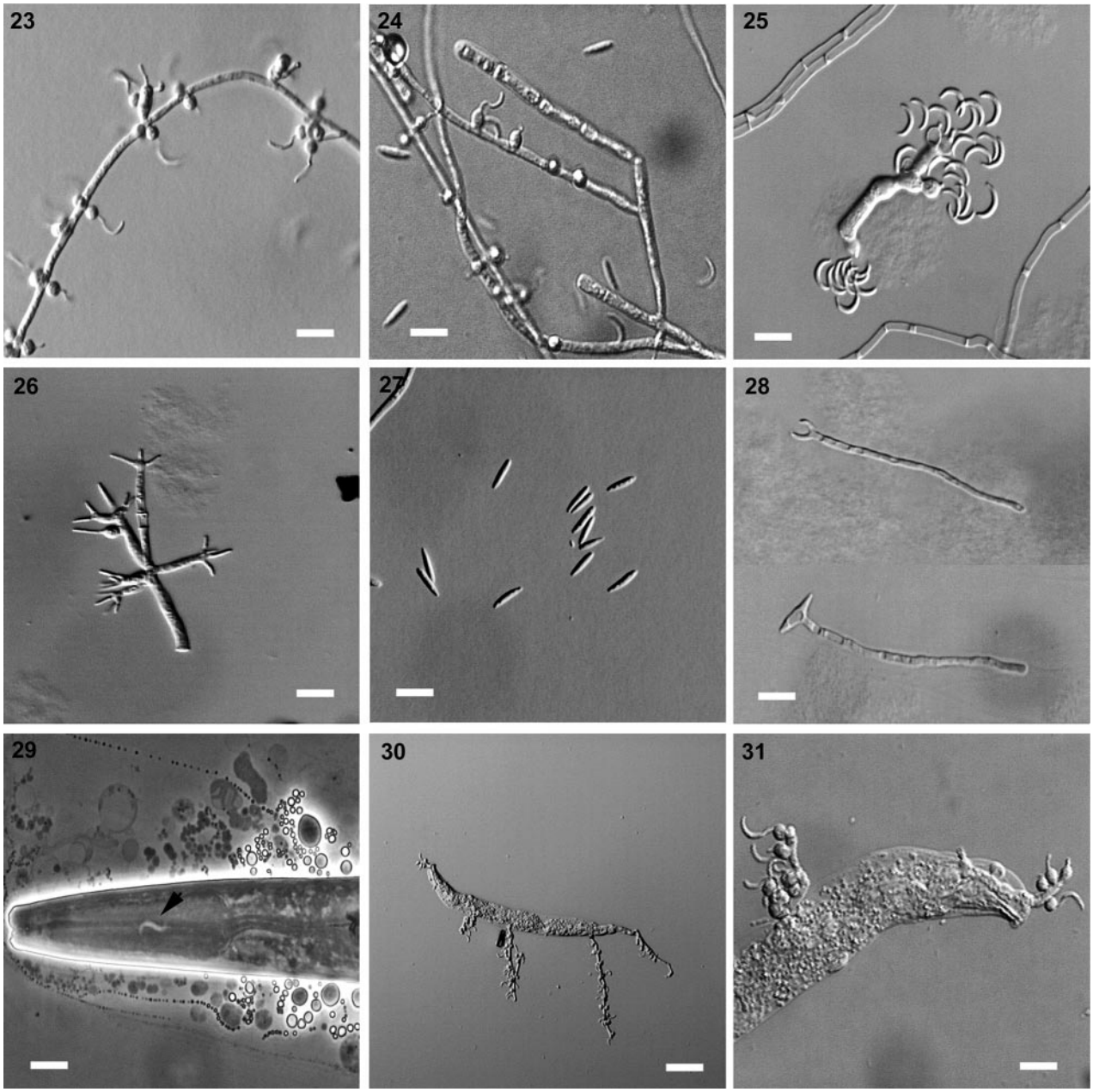
2. Podocrella harposporifera (Samuels) Chaverri et Samuels, comb. nov. FIGS. 10, 11

≡ *Atricordyceps harposporifera* Samuels, New Zealand J Bot 21:174. 1983.

Anamorph: *Harposporium* sp.

Stromata solitary, black, stipitate-capitate, ca. 7 mm diam, upper surface of cap strongly tuberculate due to protruding perithecia. Stroma outer tissue *textura angularis,* cells thin-walled; inner tissue *textura intricata,* hyphae thin-walled. Perithecia ovoid, 470–590 × 250–330 μm. Ascospores multiseptate, hyaline, smooth, disarticulating at middle septum; part-ascospores lanceolate, multiseptate, (44.0–)48.5–57.5(–68.0) × 2.0–2.5 μm.

Colonies on CMD (Difco cornmeal-dextrose agar) as first white, then becoming pale yellow; on PDA (Difco potato-dextrose agar) first white, then becoming sulphur yellow, then olivaceous. Phialides globose to ampulliform, ca. 4 μm diam with an apical elongation or neck 3–4 × 1 μm. Conidia hyaline, smooth,



FIGS. 23–31. Anamorph of *Podocrella peltata* ARSEF 5410. 23–25. Conidiophores and conidia of the *Harposporium* anamorph. 24, 25. Arthroconidia forming *Harposporium* conidiogenous cells and conidia upon germination. 26, 27. *Hirsutella*-like synanamorph. 26. Conidiophore. 27. Conidia. 28. Germ tube arising from the center of the conidium. 29–31. Infected nematodes. 29. Conidium inside the esophagus of nematode (arrow). 30, 31. Nematode cadavers bearing erect *Harposporium* conidiophores and conidia. Bars: 23–29, 31 = 10 μm; 30 = 20 μm.

arcuate, distance across between two ends (7–)8–12(–13) μm, 1–2 μm wide (anamorph description based on Samuels 1983).

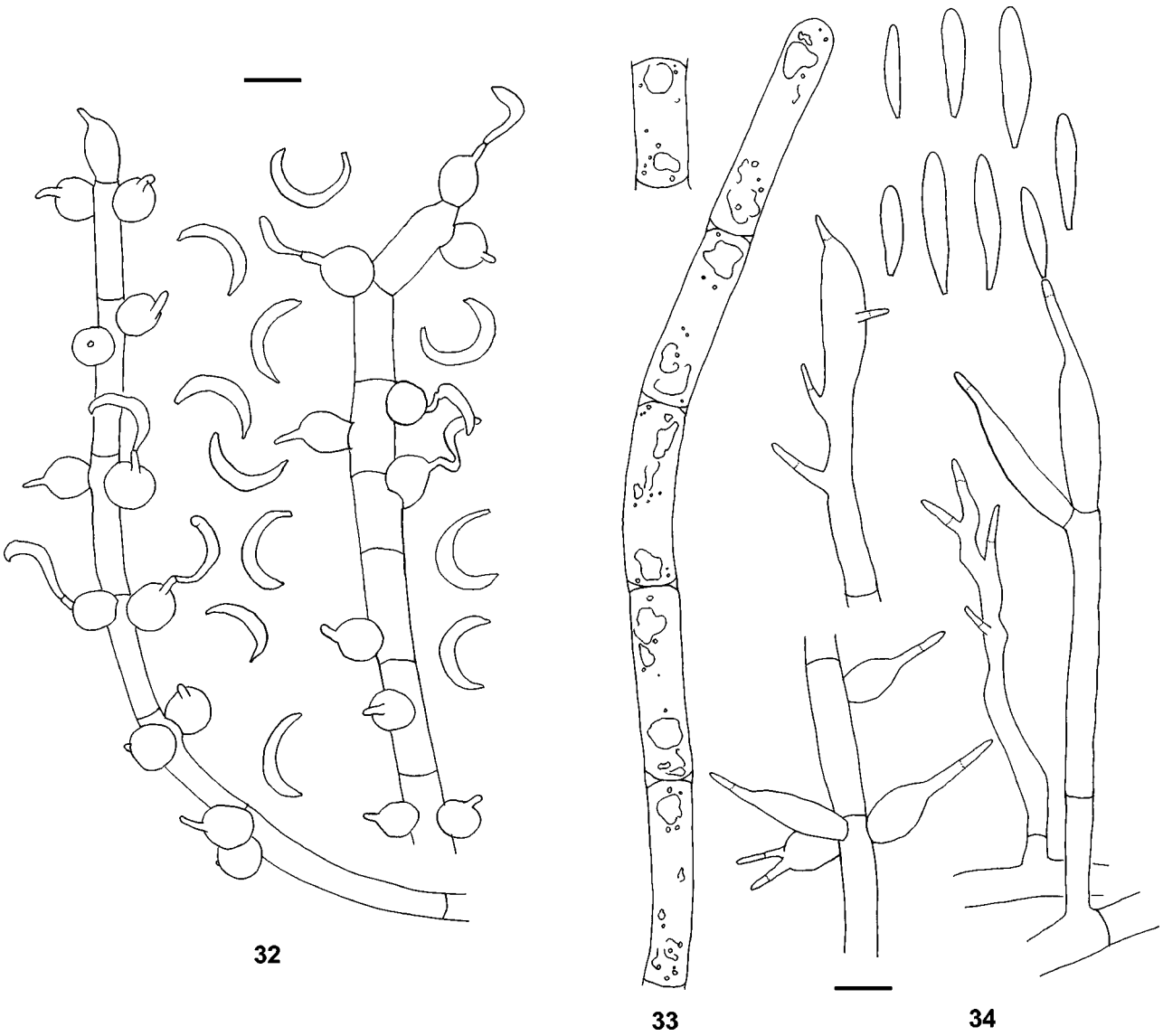
Descriptions and illustrations. Samuels (1983) FIGS. 1, 2

Habitat. Possibly growing on an arthropod.

Known distribution. New Zealand (type locality).

Holotype. NEW ZEALAND. AUCKLAND: Waitakere Ranges, Waitemata City, Fairy Falls Track, off Mountain Road, on an arthropod, 12 Aug 1981, *G.J. Samuels, P.R. Johnston, R.E. Beever, R.P. Korf, J.W. Paden* (PDD 42208!; culture G.J.S. 81-358 = ARSEF 5472).

Notes. Samuels (1983) believed that the anamorph of *P. harposporifera* could be attributed to *Harposporium anguillulae.* However, based on colony characteristics, conidial measurements, and LSU sequences (FIG. 1), we conclude that the anamorph of *P. har-*



FIGS. 32–34. Anamorph of *P. peltata* ARSEF 5410. 32. *Harposporium* conidiophores and conidia. 33. Arthroconidia. 34. *Hirsutella*-like conidiophores and conidia. Bars = 5 μm.

posporifera is possibly an undescribed species of *Harposporium*.

3. Podocrella peltata (Wakefield) Chaverri et K.T. Hodge, comb. nov. FIGS. 12–18, 23–34

≡ *Cordyceps peltata* Wakefield, Bull Misc Inform Kew, p. 74. 1916.

≡ *Ophiocordyceps peltata* (Wakefield) Petch, Trans Br Mycol Soc 16:74. 1931.

≡ *Wakefieldiomyces peltatus* (Wakefield) Y. Kobayasi, Bull Natl Sci Mus Tokyo, B. 7:2. 1981.

Anamorph: *Harposporium* sp.

Stromata solitary, dark brown, stipitate-capitate, upper surface of cap tuberculate due to protruding perithecia, cap circular in outline, 3–4.5 mm diam (n = 6). Stroma outer tissue *textura angularis,* cells thin-walled. Stroma inner tissue *textura intricata*, hyphae thin-walled. Perithecia obpyriform, (455–)489–519(–532) × (177–)186–200(–211) μm (n = 10). Ascospores multiseptate, hyaline, smooth, disarticulating at the middle septum; part-ascospores lanceolate, multiseptate, (36.5–)41.5–47.2(–57.5) × (2.7–)3.5–4.2(–5.2) μm.

Colonies developing slowly on MEA, reaching 11 mm diam after 2 wk at room temperature. Initially white to peachy cream, becoming pale yellow-puce in the center, symmetrical, mycelium fairly closely appressed to the medium, reverse pale olivaceous brown in central zone. Conidiophores present throughout surface mycelium, more or less erect, septate, each cell bearing one whorl of 2–4 conidiogenous cells just below the distal septum, terminat-

ing apically in one or more conidiogenous cells. Conidiogenous cells phialidic, subglobose to ampulliform, 3.5–4.5(–6.0) × 3.0–4.0(–4.5) μm, with a narrow cylindrical neck (0.5–)1.2–2.5 × 0.5–1.0 μm, which rarely branches to form a second short conidiogenous neck. Conidia produced enteroblastically, hyaline, arcuate and slightly helically twisted (when seen out of plane), distal end with a slight asymmetrical beak, proximal end narrowly truncate. Average conidial length, measured as linear distance covered (not circumference) 5.5–7.5 × 0.9–1.3 μm, diameter at widest point 0.9–1.3 μm. ***Hirsutella*-like synanamorph** observed in culture: conidiophores erect, loosely branched at acute angles. Conidiogenous cells phialidic, (8–)16–22(–30) × 2.0–3.1 μm, awl-shaped or with somewhat inflated base tapering gradually into a narrow neck that typically contains refractive apical thickening. Additional necks (4.0–5.5 μm) occasionally produced on same conidiogenous cell or on a subtending cell. Conidia (7.7–)8.0–11.8(–13.5) × 1.2–2.5 μm, hyaline, acerose, straight or slightly curved, with truncate narrow base and rounded apex, lacking mucilaginous coat. **Arthroconidial synanamorph** also observed in culture: Conidiophores erect, diverging apically to form 2–6 hyphal branches that divide internally to produce 2–8 stout cylindrical arthroconidia (8.5–)12.0–160.0(–20.5) × 3.5–4.4 μm with refractive contents and slightly thickened walls.

Habitat. Possibly growing on an arthropod.

Known distribution. West Indies (St. Vincent) and Costa Rica.

Specimens examined. COSTA RICA. PUNTARENAS: Parque Internacional La Amistad, Las Alturas Biological Station, elev. 1580 m, 8°57′00″N, 82°50′00″W, Trail to Cerro Echandi, on arthropod, 6 May 1996, *S.M. Huhndorf (2243A), F. Fernández* (BPI 749196!; culture ARSEF 5410 = G.J.S. 96-242). WEST INDIES. St Vincent, parasitic on larvae of *Cryptorhynchys* sp. (Coleoptera), infesting cultivated *Codiaeum,* 25 Jun 1915, *W.N. Sands (No. V. 25-6-15)* (HOLOTYPE. K 122466!).

Notes. Podocrella peltata can be distinguished from *P. poronioides* and other *Podocrella* species by the less tuberculate stromatal cap and thicker ascospores. In addition the stroma of *P. poronioides* is dark green to black, darker than that of *P. peltata.* Both species have been found in the West Indies. The anamorph of *P. peltata* most closely resembles *Harposporium lilliputanum* S.M. Dixon in the size of its arcuate conidia and the lack of chlamydospores in the host. Wood (1973) reported slightly smaller conidia in an isolate of *H. lilliputanum* from New Zealand. Glocking and Shimazu (1997) have reported similar *Hirsutella*-like and arthroconidial synanamorphs in a Japanese isolate of *H. lilliputanum,* however the *Hirsutella*-like anamorph they observed produced significantly shorter conidia (3–6 μm), whereas in *P. peltata* they measure 8–12 μm. The larger *Harposporium*- and *Hirsutella*-like conidia suggest that the *P. peltata* anamorph is allied but not conspecific with *H. lilliputanum.* Further work is needed to refine species concepts among *Harposporium* isolates with simple arcuate conidia, including *H. lilliputanum* and *H. anguillulae.*

4. *Podocrella poronioides* Seaver, Mycologia 20:57. 1928. FIGS. 19–22

Anamorph: Unknown.

Stromata solitary, dark green almost black, stipitate-capitate, upper surface of cap strongly tuberculate due to protruding perithecia, cap circular in outline, 3–4 mm diam (n = 5). Stroma outer tissue *textura angularis,* cells thin-walled. Stroma inner tissue *textura intricata,* hyphae thin-walled. Perithecia obpyriform, ca. 550–850 × 250–300 μm. Ascospores multiseptate, hyaline, smooth, disarticulating at middle septum; part-ascospores lanceolate, multiseptate, (37.0–)41.5–45.7(–52.2) × (2.5–)2.7–3.2(–3.7) μm.

Descriptions and illustrations. Seaver (1928), Plate 8: FIGS. 1–3; Rossman et al (1999), Plate 52 a–f.

Habitat. Possibly growing on an arthropod.

Known distribution. Trinidad (type locality).

Specimens examined. TRINIDAD. Vicinity of Valencia, possibly on arthropod buried on rotten wood, 4 Mar 1921, *F.J. Seaver (3017)* (HOLOTYPE. NY!).

KEY TO SPECIES OF *PODOCRELLA*

1. Stromata dark green to almost black, 3–7 mm diam 2
1′. Stromata dark brown, 3–4.5 mm diam 3
2. Stromata ca. 7 mm diam; part-ascospores 48.5–57.5 × 2.0–2.5 μm; known only from New Zealand . . . . . 2. *P. harposporifera*
2′. Stromata 3–4 mm diam; part-ascospores 41.5–45.7 × 2.7–3.2 μm; known only from Trinidad . . . . . 4. *P. poronioides*
3. Part-ascospores 41.5–47.2 × 3.5–4.2 μm . . 3. *P. peltata*
3′. Part-ascospores 56.7–62.2 × 2.7–3.0 μm . . . 1. *P. fusca*

ACKNOWLEDGMENTS

We thank Dr Amy Y. Rossman for the comments on this manuscript. We also are grateful to Dr Joseph W. Spatafora for access to unpublished sequences. The fieldwork in Costa Rica was financially supported by the National Biodiversity Institute (INBio) in Costa Rica as part of the effort to inventory the fungi of Costa Rica, a project financed by Global Environment Facility (GEF) through World Bank.

LITERATURE CITED

Aoki Y, Saikawa M. 1992. Arthroconidia in *Harposporium diceraeum.* Nova Hedwigia 55:479–483.

Barron GL. 1972. Nematophagous Hyphomycetes: a new species of *Harposporium*. Antonie van Leewenhoek J Microbiol Serol 38:217–222.

———. 1977. Nematophagous fungi: a new *Harposporium* parasitic on *Prismatolaimus*. Can J Bot 55:892–895.

Drechsler C. 1968. A new nematode-destroying *Harposporium*. Amer J Bot 55:1251–1253.

Esser RP, El-Gholl NE. 1992. *Harposporium,* a fungus that parasitizes and kills nematodes utilizing conidia swallowed by or sticking to its prey. Florida Dept of Agriculture, Division of Plant Industry, Nematology Circular 200.

Gams W, Zare R. 2003. A taxonomic review of the clavicipitaceous anamorphs parasitizing nematodes and other microinvertebrates. In: White JF et al, eds. Clavicipitalean Fungi. Evolutionary Biology, Chemistry, Biocontrol, and Cultural Impacts. New York: Marcel Dekker Inc. p 17–73.

Glockling SL, Shimazu M. 1997. Culturing of three species of endoparasitic fungi infecting nematodes. Mycol Res 101:55–60.

Hodge KT, Viaene NM, Gams W. 1997. Two *Harposporium* species with *Hirsutella* synanamorphs. Mycol Res 101: 1377–1382.

Huelsenbeck JP. 2000. MrBayes: Bayesian inferences of phylogeny (software). New York: University of Rochester.

Kobayashi Y. 1981. Revision of the genus *Cordyceps* and its allies 1. Bull Natl Sci Mus Tokyo 7:2–13.

Kornerup A, Wanscher JH. 1978. Handbook of colour. 3rd ed. London: Methuen London Ltd. 252 p.

Mau B, Newton M, Larget B. 1999. Bayesian phylogenetic inference via Markov Chain Monte Carlo methods. Biometrics 55:1–12.

Petch T. 1931. Notes on entomogenous fungi. Trans Br Mycol Soc 16:55–75.

Posada D, Crandall KA. 1998. Modeltest: testing the model of DNA substitution. Bioinformatics 14:917–918.

Rannala B, Yang ZH. 1996. Probability distribution of molecular evolutionary trees: A new method of phylogenetic inference. J Molec Evol 43:304–311.

Rossman AY, Samuels GJ, Rogerson CT, Lowen R. 1999. Genera of Bionectriaceae, Hypocreaceae and Nectriaceae (Hypocreales, Ascomycetes). Stud Mycol 42:1–248.

Samuels GJ. 1983. Ascomycetes of New Zealand 6. *Atricordyceps harposporifera* gen. et sp. nov. and its *Harposporium* anamorph. New Zealand J Bot 21:171–176.

Seaver FJ. 1928. Studies in tropical Ascomycetes IV. Some Hypocreales from Trinidad. Mycologia 20:52–59.

Shimazu M, Glockling SL. 1997. A new species of *Harposporium* with two spore types isolated from the larva of a cerambycid beetle. Mycol Res 101:1371–1376.

Sung G-H, Spatafora JW, Zare R, Hodge KT, Gams W. 2001. A revision of *Verticillium* sect. *Prostrata*. II. Phylogenetic analyses of SSU and LSU nuclear rDNA sequences from anamorphs and teleomorphs of the Clavicipitaceae. Nova Hedwigia 71:311–328.

Swofford D. 1999. PAUP*: Phylogenetic Analysis Using Parsimony (*and other methods), version 4. Sunderland, Massachusetts: Sinauer Associates.

Thompson JD, Gibson TJ, Plewniak F, Jeanmougin F, Higgins DG. 1997. The Clustal X windows interface: flexible strategies for multiple sequence alignment aided by quality analysis tools. Nucl Acids Res 24:4876–4882.

Viaene NM-M. 1996. Damage threshold and biological control of the northern root-knot nematode (*Meloidogyne hapla* Chitwood) infecting lettuce (*Lactuca sativa* L.) in organic soil. [Doctoral dissertation]. Cornell University.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246.

Wakefield EM, Groves WB. 1916. Fungi Exotici: XX. Bull Misc Inform Kew 1916:71–77.

Wood FH. 1973. Nematode-trapping fungi from a tussock grassland soil in New Zealand. New Zealand J Bot 11: 231–240.