MYCOLOGICAL SURVEY OF RÍO CAMUY CAVES PARK, PUERTO RICO

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The Río Camuy Caves Park is part of the Río Camuy Cave System in the northern karst belt of Puerto Rico. Thirty-nine fungal species and 11 cellular and plasmodial slime molds are new records for caves and sinkholes of the Río Camuy Cave Park. Two of the cellular slime molds Dictyostelium citrinum *and* D. macrocephalum *are new records for Puerto Rico.*

El Parque de las Cavernas del Río Camuy pertenece al Sistema de Cavernas del Río Camuy en el cinturón del carso norteño de Puerto Rico. Se informan 39 especies de hongos y 11 hongos mucilaginosos celulares y plasmodiales como nuevos registros para cuevas y sumideros del Parque de las Cavernas del Río Camuy. Dos de los hongos mucilaginosos celulares Dictyostelium citrinum *y* D. macrocephalum *son nuevos registros para Puerto Rico.*

Fungi play roles in cave communities as saprotrophs or pathogens. Fungi affect the population dynamics of cave biota and contribute in the decomposition of organic matter, making it available to other members of the cave community. Diets of many insect, including cavernicoles, include fungal spores and mycelia (Culver 1982), and spores from at least 15 fungal genera probably constitute an important element in the diet of springtails (Collembola: Insecta) (Cubbon 1976; Culver 1982; Roselló *et al.*, 1986). Insects are considered an effective dispersion vector of fungal spores and other microorganisms because of nonspecific factors in attachment to host insect cuticles (Boucias *et al.* 1988). Chiropterans, insects, and rodents transport many fungal species to this bat-guano enriched soil environments (Hoff & Bigler 1981).

The Río Camuy Cave System [RCCS] is a 15-km long, subtropical karst feature carved by the Camuy River in the subtropical moist forest (Ewel & Whitmore 1973) of northern Puerto Rico. This system, one of the largest caves in Puerto Rico, is part of the National Parks Company of the Commonwealth of Puerto Rico natural protected area. In the 1960s, Russell and Jeanne Gurnee described the limestone features, caves, conservation, and future development, of what is now Río Camuy Caves Park [RCCP] (Gurnee 1967; Gurnee & Gurnee 1974, 1987; Martínez-Oquendo 1983) (Fig. 1). The geology and hydrology of the RCCS were treated by Thrailkill (1967), Monroe (1976, 1980), Torres-González (1983), Troester and White (1986), and Troester (1988, 1994). A recent study (Lugo *et al.* 2001) summarized what is known about the karst of Puerto Rico (including RCCS), including its importance and anthropogenic impact. Bat-guano enriched soil was formerly mined from Puerto Rican caves (Cadilla 1962; Beck *et al.* 1976; Campbell 1994; Frank 1998), but the guano in Río Camuy caves apparently was not heavily exploited (Cardona-Bonet, pers. comm. 2001).

In general, the rainy season in Puerto Rico peaks in May-June and August-September. Air temperatures in RCCS range from 18-25° C (Mercado, pers. comm. 2000), relative humidity from 88-100% (Troester 1988), and the average annual rainfall for Lares, 7 km SW of RCCP, is 2435 mm.

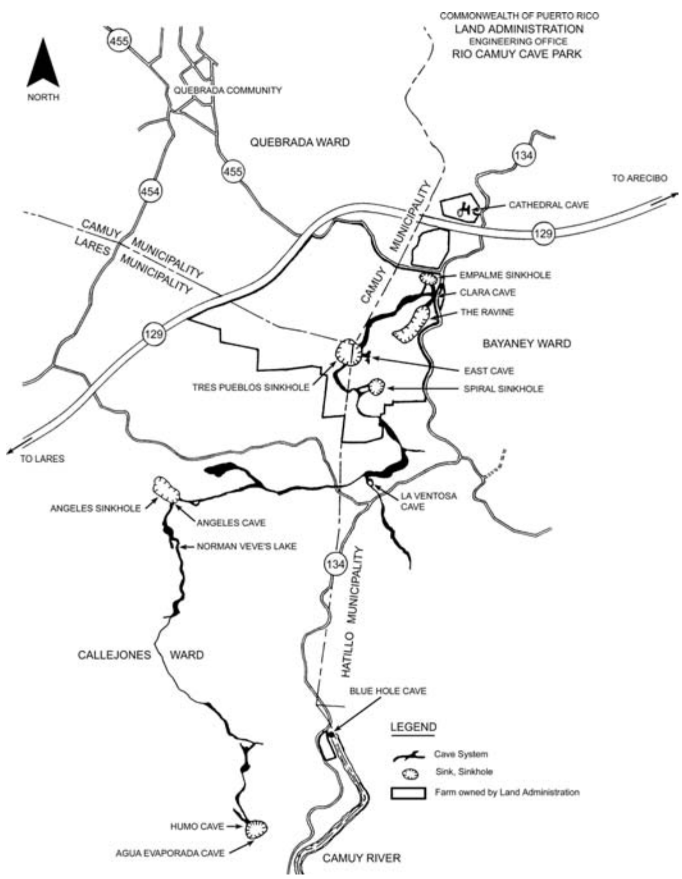


Figure 1. Map of Río Camuy Caves Park. Redrawn after Luis Ayala (November 1988) by permission (National Parks Company of the Commonwealth of Puerto Rico), based on Gurnee & Gurnee (1974) original map.

The vascular flora of the sinkholes and mogotes of RCCP was surveyed by Del Llano (1982) and Vives *et al.* (1985), recording 136 and 73 plant species, respectively. Reyes-Colón (1999) and Reyes-Colón and Sastre-DJ (2000) recorded 50 species: 31 mosses (11% of the moss flora of Puerto Rico) and 19 liverworts (8% of the liverwort flora of Puerto Rico) from 2 sinkholes (Espiral and Empalme Sinks) in RCCP.

The caves and sinkholes of RCCP are inhabited by a variety of invertebrates and vertebrates (Sullivan 1966, 1967; Peck 1974, 1981, 1994; Díaz-Díaz 1982, 1983; Santos-Flores 2001; Lugo *et al.* 2001). Multispecies assemblages of bats are reported from RCCP, as is common in many Puerto Rican caves (Rodríguez-Durán & Lewis 1987; Conde Costas & González 1990; Rodríguez-Durán 1998). With the exception of the endemic amphipod *Alloweckelia gurneei* Holsinger & Peck (Holsinger & Peck 1967) and the cave cockroach *Nelipophygus* sp. (Peck 1981), no other troglobites have been reported from RCCS.

Betancourt *et al.* (1988) recorded 8 genera of mitosporic fungi (*Aspergillus* sp., *Cladosporium* sp., *Curvularia* sp., *Fusarium* sp., *Geotrichum* sp., *Scopulariopsis* sp., *Sepedonium* sp., and *Rhizopus* sp.) from Ensueño Cave (3 km south of RCCP). Eight species of aquatic hyphomycetes (*Brachyosphaera jamaiciensis* [Crane & Dumont] Descals, *Clavariopsis azlanii* Nawawi, *Dendrosporium lobatum* Plakidas & Edgerton: Crane, *Isthmolongispora quadricellularia* Matsushima, *Tricladiospora brunnea* [Nawawi] Nawawi & Kuth., *Tripospermum* sp., *Varicosporeum giganteum* Crane, *Xenosporium berkeleyi* [Curtis] Pirozynski) were also isolated in river foam from Tres Pueblos Sinkhole (Santos-Flores & Betancourt-López 1997) in RCCP.

This report summarizes a mycological survey of the bat-guano enriched soil, leaf litter, wood, top soil, streams, and intermittent pools in RCCP.

Methods

I conducted a basic inventory of the fungi, and the cellular/plasmodial slime molds at Cathedral Cave, Clara Cave, Empalme Sinkhole, and Tres Pueblos Sinkhole (RCCP, Bayaney topographic quadrangle) (Figure 1). These sites are described below.

•Cathedral Cave (“Cueva La Catedral”) (18°21’04.2” N, 66°48’99.3” W), a hydrological inactive cave, is located adjacent to the hydrographic basin of the Río Camuy, at the north end of RCCP (Fig. 1). This cave is 122 m long, with 2 main parallel galleries (or chambers), which run north-south and are connected by a short passage, with 2 natural skylights, about 24 and 38 m above the floor of the cave. The west hall contains rock art of presumed aboriginal origin (42 pictographs in total; Nieves-Rivera, unpubl. data).

•Clara Cave [“Cueva Clara de Empalme”, “Empalme Cave” of Peck (1974)] (18°20’85.4” N, 66°49’16.0” W) is located south of Cathedral Cave (Fig. 1). This cave is part of the ancient course of the Río Camuy and is higher than the present river level. The main passage is a diagonal tunnel 115 m long and 52 m high. A secondary passage extends 30 m to the west. The primary entrance is shown in Figure 2A; there is also a vertical shaft entrance at Empalme Sinkhole. Previous hydrological studies of 2 sites (streamlet/pool R-1 [Fig. 2B] and pool R-2, both intermittent) of Cueva Clara reported concentrations of 7.9-8.6 mg/L of dissolved oxygen at 22°C, and 0.131 to 0.040 mg/L of total phosphorous, respectively (Díaz-Díaz 1982). Pool water pH has been measured between 9.1 and 9.2 with conductivity rendering 200 μ (Nieves-Rivera, unpubl. data). Fecal coliform counts have been reported as 1160 cells/mL for R-1 and 1810 cells/mL for R-2, respectively, indicating a runoff water with high organic input (fecal matter and detritus) (Díaz-Díaz 1982).

•Empalme Sinkhole (“Sumidero de Empalme”) (18°20’79.9” N, 66°49’11.2” W) is a 99-m wide doline with a 125-m deep vertical shaft, connecting directly to Clara Cave (Fig. 1) and to the conical-shaped “Cueva Alta del Norte” (Fig. 2C). At the bottom, and along the upper reaches of the Río Camuy, numerous collapsed blocks are present, along with top soil composed of clay or dirt, leaf litter, mosses, and rocks with lichens. Further description of this doline was given by Gurnee (1967), Gurnee & Gurnee (1974), Reyes-Colón (1999), and Reyes-Colón & Sastre-DJ (2000).

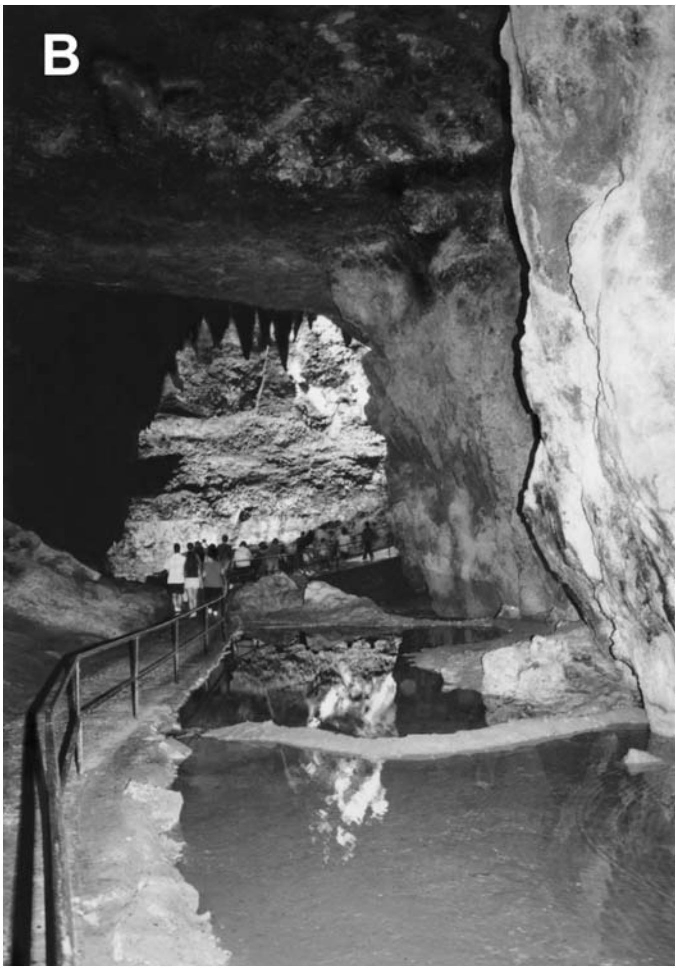
•Tres Pueblos Sinkhole (“Sumidero Tres Pueblos”) (18°20’30.5” N, 66°49’26.3” W) is a doline in RCCP, and was described by Gurnee (1967) and Gurnee & Gurnee (1974).

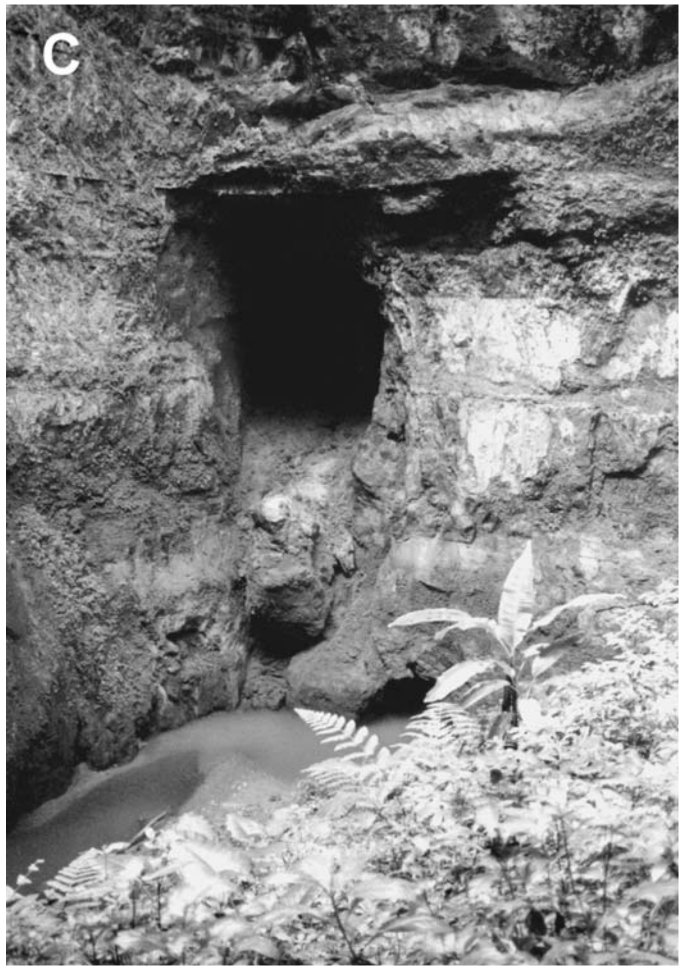
Field work

Field work at Cathedral Cave, Clara Cave, and Empalme Sinkhole was carried out on 8 August 1999, supplemented by river water and mud samples collected from Tres Pueblos Sinkhole in May 1993. Undergraduate and graduate students, and professors from the University of Puerto Rico at Mayagüez Campus (Departments of Crop Protection and Marine Sciences) participated in the survey.

Laboratory work

Fungi were isolated from field-collected samples. Samples of bat-guano enriched soil, leaf litter, wood, water, and soil were collected using sterile plastic bags (Whirl Pak). Higher fungi (ascomycetes and basidiomycetes) were collected and processed following Nieves-Rivera *et al.* (1999). Bat-guano enriched soil samples were processed according to Orpurt (1964), Gaur and Lichtwardt (1980), and Rutherford and Huang (1994). Freshwater fungi sampling followed Sparrow (1960), Nieves-Rivera and Betancourt-López (1994) and Santos-Flores and Betancourt-López (1997). Potato dextrose agar acidified with 10% lactic acid, Rose Bengal agar, and Sabouraud’s dextrose agar were used as fungal growth media. Wet mount observations were made with a Nikon Labophoto-2 microscope. All voucher specimens were taken to the Center for Forest Mycology (U.S. Department of Agriculture, Forest Products Laboratory, Sabana Station, Puerto Rico) where they are being prepared for deposition in the herbarium of the Department of Natural Sciences, University of Puerto Rico





Figures 2A-C. Río Camuy Caves Park study sites. A. Main entrance of Clara Cave. B. Intermittent streamlet R-1 seen from inside Clara Cave. C. Inside Empalme Sinkhole (with a view of Cueva Alta del Norte), mesophytic forest, Río Camuy is at the bottom.

Piedras, Puerto Rico.

RESULTS AND DISCUSSION

A total of 45 true-fungi (39 new records) and 16 fungal-like organisms (11 Dictyosteliomycota/Myxomycota and 5 Oomycota; all new records) are reported from RCCS. Of these, a total of 36 taxa (34 micromycetes and 2 macromycetes) from 17 genera were isolated from cave bat-guano enriched soil or topsoil (Table 1). *Aspergillus*, *Penicillium*, *Fusarium*, *Cladosporium*, *Trichoderma*, *Paecilomyces*, *Curvularia*, *Gliocladium*, *Hirsutella*, and *Sepedonium* were the most common hyphomycete genera in cave soil (Table 1). All these genera are dematiaceous hyphomycetes. *Mycelia Sterilia sensu* Rutherford & Huang (1994) was also present in cave soil. Mitosporic fungi isolated in this study were present as spores, actively growing mycelium or as resting mycelial cysts. Other frequently isolated fungi were members of the Zygomycota, *Mucor*, *Rhizopus*, and an undetermined entomophylic species. The pathogen *Histoplasma capsulatum* Darling, the causative agent of histoplasmosis, was not isolated from cave samples, but was found in 1979 in Humo Cave, part of RCCS (Carvajal-Zamora, pers. comm. 1998; Table 1).

The habitat below the skylights and at other openings of RCCS differs little from the surface (Gillieson 1996). Outside of the study area, *Xylaria* (Ascomycota) and *Lepiota* (Basidiomycota) have been found in caves below skylights in Espinar Cave (Mona Island), De los Santos/Braceros Cave

Table 1. Summary of the mycobiota of the Río Camuy Cave System, Puerto Rico. Nomenclature is based on Hawksworth *et al.* (1995), Alexopoulos *et al.* (1996), and Lodge (1996).

TAXON	FAMILY	SUBSTRATE [1]	REFERENCES [2]
FUNGI [= MYCOTA]			
HYPHOMYCETES [= MITOSPORIC FUNGI]			
Aspergillus flavus Link	Anamorphic (A.) Trichocomaceae	S	2, 5, 7
Aspergillus fumigatus Fresenius	A. Trichocomaceae	S	2, 5, 7
Aspergillus nidulans (Eidam) G. Winter	A. Trichocomaceae	S	2, 5
Aspergillus niger var. *niger* van Tieghem	A. Trichocomaceae	S	1, 2, 4, 5, 7
Brachiosphaera jamaiciensis (Crane & Dumont) Descals	——————————-	S	6
Cladosporium cladosporoides (Fresenius) deVries	A. Mycosphaerellaceae	D, S, W	2, 5, 7
Cladosporium herbarum (Pers.) Link	A. Mycosphaerellaceae	S, D	1, 2, 5, 7
Clavariopsis azlanii Nawawi	——————————-	L, R	6
Curvularia sp.	A. Pleosporaceae	S	1, 5
Dendrosporium lobatum Plakidas & Edgerton: Crane	——————————-	L, R	6
Fusarium cf. *oxysporum* Schl.	A. Hypocreaceae	S	1, 2, 5, 7
Fusarium solani (Martius) Sacc.	A. Hypocreaceae	S	5, 7
Geotrichum sp.	A. Dipodascaceae	S	1, 5
Gliocladium cf. *roseum* Bain.	——————————-	S	2, 5
Hirsutella sp.	——————————-	I	2, 5
Histoplasma capsulatum Darling [3]	A. Onygenaceae	S	3, 7
Isthmolongispora quadricellularia Matsushima	——————————-	L, R	6
Neurospora crassa Shear & Dodge	Sordariaceae	S	2, 5, 7
Paecilomyces sp.	A. Trichocomaceae	S	2, 5
Penicillium cf. *crysogenum* Thom	——————————-	S	2, 5, 7
Penicillium lilacinum Thom	——————————-	S	2, 5, 7
Penicillium roqueforti Thom	——————————-	S	2, 5
Penicillium variable Westling	——————————-	S	2, 5
Scopulariopsis sp.	A. Microascaceae	S	1
Sepedonium sp.	A. Hypocreaceae	S	1, 5
Tricladisopora brunnea (Nawawi) Nawawi & Kuth.	——————————-	L, R	6
Trichoderma koeningii Oudermans	A. Hypocreaceae	S	2, 5
Trichoderma viridae Pers.: Fr.	A. Hypocreaceae	S	2, 5, 7
Tripospermum sp.	A. Capnodiaceae	L, R	6
Varicosporium giganteum Crane	——————————-	L, R	6
Xenosporium berkeleyi (Curtis) Pirozynski	——————————-	L, R	6
Mycelia Sterilia	Agonomycetaceae	S, W, I	2, 5
ASCOMYCOTA			
Xylaria polymorpha (Pers.: Mérat) Grev.	Xylariaceae	W	2, 5, 7
Xylaria sp.	Xylariaceae	W	2, 5
BASIDIOMYCOTA			
Cotylidia aurantica (Pers.) A. L. Welden	Podoscyophaceae	W	8
Geastrum cf. *saccatum* (Fr.) Ed. Fischer [4]	Geastraceae	S	5
Lepiota sp.	Agaricaeace	S	2, 5
Marasmius cf. *atrorubens* (Berk.) Berk.	Tricholomataceae	L	5
Polyporus tenuiculus (Beauv.) Fr. [5]	Polyporaceae/Coriolaceae	W	5
Ramaria sp.	Ramariaceae	L	8
Rigidoporus microporus (Fr.) Overeem.	Polyporaceae/Coriolaceae	W	8
ZYGOMYCOTA			
Mucor sp.	Mucoraceae	D, S, W	2, 5
Rhizopus stolonifer (Ehr.: Fr.) Vuill.	Mucoraceae	D, S	1, 2, 5, 7
Undetermined species	Mucoraceae	I	5
CHYTRIDIOMYCOTA			
Rhizophydium sp.	Chytridiaceae	P	5
CHROMISTA [= STRAMINIPILA]			
OOMYCOTA			
Achlya americana Humphrey	Saprolegniaceae	R	4, 5, 7
Achlya parasitica Coker	Saprolegniaceae	R	4, 5
Apodachlya sp.	Leptomitaceae	R	4, 5
Brevilegnia sp.	Saprolegniaceae	R	4, 5
Saprolegnia sp.	Saprolegniaceae	R	4, 5

PROTOZOA			
DICTYOSTELIOMYCOTA			
Dictyostelium aureostipes Cavender, Raper & Norberg [6]	Dictyosteliaceae	L, S	4, 5
Dictyostelium citrinum Vadell, Holmes & Cavender [6]	Dictyosteliaceae	L, S	4, 5
Dictyostelium giganteum Singh [6]	Dictyosteliaceae	L, S	4, 5
Dictyostelium macrocephalum Hagiwara, Yeh & Chien [6]	Dictyosteliaceae	L, S	4, 5
Dictyostelium mucoroides Brefeld [6]	Dictyosteliaceae	L, S	4, 5
Dictyostelium purpureum Olive [6]	Dictyosteliaceae	L, S	4, 5
Polysphondylium pallidum Olive [6]	Dictyosteliaceae	L, S	4, 5
Polysphondylium violaceum Brefeld [6]	Dictyosteliaceae	L, S	4, 5
MYXOMYCOTA			
Arcyria cf.	Arcyriaceae	B, W	4, 5
Comatricha cf.	Stemonitidaceae	L	5
Stemonitis cf. *herbatica* Peck	Stemonitidaceae	W	4, 5, 7

[1] Substrates: B = Bat bones; D = Dung; I = Dead insects; L = Leaf litter; P = Pollen; R = River water or foam; S = Bat-guano enriched soil or top soil; W = dead wood. The substrates were the location where the fungus was found growing or just the site from which a spore was isolated.

[2] References: 1 = Betancourt *et al.* (1988); 2 = Carvajal-Zamora & Nieves-Rivera (1998); 3 = Lewis (1989); 4 = Nieves-Rivera & Carvajal-Zamora (2000); 5 = Nieves-Rivera (present work); 6 = Santos-Flores & Betancourt-López (1997); 7 = Stevenson (1975); 8 = D. J. Lodge (pers. comm. 2001).

[3] Carvajal-Zamora (pers. comm. to Norman Veve Carreras [SEPRI] in 1979 and to me in 1998) isolated this pathogen from bat-guano of Humo Cave (= Cueva Humo) in 1979.

[4] *Geastrum* cf. *saccatum* was collected directly from top soil (not in bat-guano enriched soil), next to the edge of Tres Pueblos Sinkhole.

[5] *Polyporus tenuiculus* (= *Favolus brasiliensis* [Fr.] Fr.) basidiomata was consumed by *Caracolus caracola* (Pulmonata: Gastropoda) in a similar way as reported by Lodge (1996).

[6] Species cultured and identified by John C. Landolt, Shepherd College, Shepherdstown, WV.

(Cabo Rojo), and Cathedral Cave (Nieves-Rivera, unpubl. data). These openings admit plant debris from which fungi sporulate and fruiting bodies may develop.

Food is, in general, relatively scarce in deeper portions of caves (Culver 1982; Gillieson 1996). Most of the fungi isolated in this study are edaphic microfungi. These fungi feed and sporulate on different substrates (e.g., a carcasses, hair, seeds, or bat-guano). Use of similar substrata by both lower and higher fungi has been reported for Diablo Cave (Mexico) (Hoffman *et al.* 1986), Hendrie River Water Cave (Michigan) (Volz & Yao 1991), West Virginia Caves (Rutherford & Huang 1994), and Kozlov Rob Cave (Slovenian) (Gunde-Cimerman *et al.* 1998).

Other fungal species recorded in RCCP, particularly in wet soil and ponds formed when the river rises, included various species of Oomycota (e.g., *Achlya americana* Humphrey, *A. parasitica* Coker, *Brevilegnia* sp., and *Saprolegnia* sp.) Nieves-Rivera & Betancourt-López (1994) isolated *Saprolegnia ferax* (Gruith.) Thuret and *Achlya americana* Humphrey from a stream pond in El Convento Cave Spring System (Guayanilla, southwestern Puerto Rico), an ancient course of Río Camuy (Troester 1988). A chytrid (*Rhizophydium* sp.) was isolated from Tres Pueblos Sinkhole in 1993 and also has been isolated from a stream (Quebrada de Oro) at Mayagüez (Nieves-Rivera, unpubl. data).

In the entrances of RCCS, several higher fungal species, *Cotylidia aurantica* (Pers.) A. L. Welden, *Rigidoporus microporus* (Fr.) Overeem., *Ramaria* sp., *Geastrum* cf. *saccatum* Fr. (Fig. 3J), *Marasmius* cf. *atrorubens* (Berk.) Berk., and *Polyporus tenuiculus* (Beauv.) Fr. [= *Favolus brasiliensis* (Fr.) Fr.] were collected. *Polyporus tenuiculus* was observed being consumed by the land snail *Caracolus caracola* L. (Pulmonata: Gastropoda). Lodge (1996) reported *C. caracola* devouring basidiocarps of *P. tenuiculus* from El Verde Long Term Ecological Research site in the Luquillo Mountains, northeastern Puerto Rico. Undetermined microfungi are dominant on bat-guano deeper in RCCS (e.g., Cathedral Cave). These mycelial mats are found in caves with bat colonies in the northern and southern karst areas of Puerto Rico (Nieves-Rivera, unpubl. data).

Myxomycetes (plasmodial slime molds) *Arcyria* cf., *Comatricha* cf., and *Stemonitis* cf. *herbatica* Peck were collected from all entrances sites, except Empalme Sinkhole. Eight species of dictyostelids (cellular slime molds) were isolated from bat-guano enriched soil, or leaf litter, including *Dictyostelium aureostipes* Cavender, Raper & Norberg, *D. citrinum* Vadell, Holmes & Cavender, *D. giganteum* Singh, *D. macrocephalum* Hagiwara, Yeh & Chien, *D. mucoroides* Brefeld, *D. purpureum* Olive, *Polysphondylium pallidum* Olive, and *P. violaceum* Brefeld (Table 1). *Polysphondylium violaceum* and an undetermined myxomycete plasmodium were previously isolated from bat-guano enriched soil from El Caballo/Pájaros Cave (Playa de Pájaros) and a bat cave (Uvero), both in Mona Island (Landolt, pers. comm. 1998; Nieves-Rivera & McFarlane 2001: map). *Polysphondylium violaceum* is fairly widespread in many tropical, subtropical, and temperate habitats (Landolt *et al.* 1992; Reeves *et al.* 2000); *P. violaceum* has been isolated from Appalachian Mountains and eastern Russia, and is widely distributed at lowlands and high altitudes – one isolate came from ~2000 m msl (Landolt, pers. comm. 2002). *Dictyostelium* and *Polysphondylium* species are inhabitants of moist forest soils and litter (Stephenson & Landolt 1996; Stephenson *et al.* 1999).

Forty-four of the 50 fungi and slime molds are new records for Cathedral Cave, Clara Cave, and Empalme and Tres Pueblos Sinkholes of RCCP (part of the RCCS). The cellular slime molds *Dictyostelium citrinum* and *D. macrocephalum* are new records for Puerto Rico.

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