Costa Rican gasteromycetes (Basidiomycota, Fungi): Calostomataceae, Phallaceae and Protophallaceae

Larissa Trierveiler-Pereira¹*, Andrew W. Wilson², Rosa Mara B. da Silveira¹ and Laura S. Domínguez³

¹ Programa de Pós-Graduação em Botânica, Depto. de Botânica, Universidade Federal do Rio Grande do Sul, 91501-970, Porto Alegre, RS, Brazil
² Chicago Botanic Garden, Plant Conservation Science, 60022, Glencoe, IL, United States of America
³ Instituto Multidisciplinario de Biología Vegetal (IMBIV), CONICET-Universidad Nacional de Córdoba, CC 495, 5000, Córdoba, CBA, Argentina

With 5 figures

Abstract: Costa Rican specimens of Calostomataceae, Phallaceae and Protophallaceae (gasteroid basidiomycetes) kept at Herbarium F were examined and identified. Eleven species belonging to seven genera were recognized: Calostoma cinnabarinum, C. lutescens (Calostomataceae), Aseroë rubra, Clathrus columnatus, Laternea pusilla, L. triscapa, Ligiella rodrigueziana, Phallus indusiatus, P. duplicatus (Phallaceae), Protubera maracuja and P. jamaicensis (Protophallaceae). Calostoma lutescens is described with larger spores than those reported for the species, but molecular data confirmed the species identification. The occurrence of Protubera species in Costa Rica is reported for the first time.

Key words: Boletales, Central America, fungal taxonomy, Neotropical mycota, Phallales.

Introduction

Although the first fungal collections from Costa Rica are from the end of the nineteenth century (Rossman et al. 1998), little attention was given to gasteroid basidiomycetes until the middle of the twentieth century. Garner (1956) believed that his reports of gasteromycetes [Lycoperdon subincarnatum Peck and Cyathus stercoreus (Schwein.)

*corresponding author: lt_pereira@yahoo.com.br
De Toni] were the first ones from Costa Rica, but previously Polakowsky (1879) and Bommer & Rousseau (1896) had reported species of *Bovista*, *Lycoperdon* and *Scleroderma*.


As a result of collaboration projects between U.S.A. and Costa Rica, many specimens of gasteroid fungi were collected by researchers of these two countries and exsiccati were deposited, among other herbaria, at Field Museum of Natural History (F), Chicago, U.S.A. In this work, we present our first data on a review of Costa Rican representatives of gasteromycetes deposited at Herbarium F.

**Material and methods**

Macroscopic characteristics of the basidiomata were examined following traditional techniques used in taxonomic studies of gasteroid fungi (Miller & Miller 1988). Colors were coded according to Kornerup & Wanscher (1978). Field notes from the exsiccati were used in the descriptions. For microscopic analysis, freehand sections of basidiomata were mounted with 5% KOH and 1% phloxine. Spore dimension includes ornamentations. Photos of scanning electronic microscopy (SEM) were conducted at 'Laboratorio de Microscopía Electrónica y Microanálisis' (LABMEM) of Universidad Nacional de San Luis (Argentina). Basidiospores were mounted on aluminium stubs, covered with gold with a standard sputter coater and then photographed with a Zeiss LEO 1450VP.

Analyzed specimens are kept at Herbarium F. Specimens from herbaria ICN and CORD (Thiers 2012) were also analyzed for comparison and confirmation of the species identifications.

Molecular analysis was performed to determine the taxonomic identity of *Calostoma lutescens* from Costa Rica. DNA extraction of *Calostoma* specimens for molecular analysis was performed using Qiagen DNeasy Plant Mini Kit (Qiagen USA, Valencia, CA. http://www.qiagen.com/). Sequences of nuclear ribosomal internally transcribed spacer regions 1 and 2 (ITS), and nuclear ribosomal large subunit (LSU) were amplified respectively using a polymerase chain reaction (PCR) with primer combinations ITS1F and ITS4 (Gardes & Bruns 1993, White et al 1990), and LR0R and LR5 (White et. al 1990). PCRs used a simple thermalcycler protocol consisting of an initial denature step at 95°C for 5 min, followed by a 32 cycles of a denature step at 95°C for 1 min, an annealing step at 50°C for 30 seconds, followed by an extension step at 72°C for 1 min. Lastly a final extension step was performed at 72°C for 5 min. PCR product was examined using gel electrophoresis and successfully amplified product was used in cycle sequencing. Cycle sequencing protocols were as follows: denature step of 94°C for 2 min; 35 cycles of 92°C for 30 seconds, 55°C for 1 min, and 72°C for 30 seconds; final extension of 72°C for 5 min. Sequencing products were run on an ABI 377 DNA sequencer (Applied Biosystems, Foster City, California).

Sequence editing was performed using CodonCode Aligner v.3.5.7 (CodonCode Corporation, Dedham, MA, http://www.codoncode.com/). ITS and LSU sequences were used as queries against GenBank using BLAST (http://blast.ncbi.nlm.nih.gov) to retrieve additional *Calostoma* sequences from GenBank for phylogenetic analysis. Molecular ITS and LSU datasets consisting of original and GenBank sequences were assembled in Mesquite (Maddison & Maddison 2008) and aligned using MUSCLE (Edgar 2004).
Maximum likelihood (ML) analyses and ML bootstrap analyses were performed on both ITS and LSU datasets using RAxML (Stamatakis 2006), which was implemented on the CIPRES web portal (Miller et al. 2009) using 1000 bootstrap replicates to generate bootstrap statistics.

Results

Molecular analysis

Sequences of ITS and LSU genes were generated for three *Calostoma lutescens* specimens from Costa Rica (Utley750, GenBank ID JX184406) and U.S.A (AM1208, GenBank ID JX184404; NY223, GenBank ID JX184405). These sequences ranged in size from 727 to 734 nucleotides. Two LSU sequences were produced from samples Utley750 (JX184408) and AM1208 (JX184407). These sequences were 930 and 1032 nucleotides in length respectively.

Datasets for ITS and LSU genes contained 29 and 27 taxa, and were 987 and 982 characters in length respectively. Phylogenetic analysis of each dataset independently produced phylogenies that were congruent at the species level (Fig. 5). These phylogenies confirm that *C. lutescens* from Costa Rica (Utley750) is con-specific with temperate North American collections of this species with 100% bootstrap support in both ITS and LSU phylogenies.

Taxonomy

**Calostomataceae**

*Calostoma cinnabarinum* Corda, Anleitung 2: 94 (1809)

*Description:* see Coker & Couch (1928); Castro-Mendoza et al. (1983).

*Distribution:* pantropical (Calonge et al. 2005a).

*Comments:* The species is characterized by short stalked and orange reddish basidiomata; gelatinous, deciduous exoperidium; and elliptical basidiospores with prominent reticulations (Castro-Mendoza et al. 1983). *Calostoma cinnabarinum* was previously reported from Costa Rica by Calonge et al. (2005a).


*Calostoma lutescens* (Schwabe) Burnap, Bot. Gaz. 23: 190 (1897) Figs 1–2

Basidiomata 2.4–5.6 cm high, solitary or gregarious, long or short stalked, 1.0–4.4 cm high × 0.6–1.5 cm wide, with a globose to depressed-globose head, 1.1–1.2 cm
high × 1.3–1.8 cm wide. Stalk, columnar, of longitudinal gelatinous-rubbery cords, branching and anastomising, dry, fibrose-cottony, semi-viscid when fresh, hard when dry, greyish yellow (2B4), in immature basidiomata continuous with the exoperidium. Exoperidium greyish yellow (1B6), ornamented with scales and pyramidal warts which are concolor or darker than exoperidium, not gelatinous, thick when dry, breaking up into small pieces and some pieces remained attached to the bottom of the head. Mesoperidium light orange (5A5) to golden yellow (5b7), horny when dry, up to 0.5 mm thick, with a reddish or concolor apical month, composed of 5–8 connected radial slits; spore-sac suspended from the peristome, thin, fragile, pale yellow (4A5). Capillitium absent; paracapillitium observed in young basidiomata, composed of hyaline to yellowish fibulate hyphae, 2.0–4.0 μm diam. Basidiospores 8.0–14 μm in diam, globose to subglobose, some flattened at the polar axis, yellowish, walls with large pits that are up to 1.0 μm diam. Stalk composed of tightly interwoven hyphae, hyaline, thick-walled and with narrow lumen, clamp connections not seen, 7.0–20 μm diam; and thin-walled, clamped hyphae, 2.5–6.0 μm diam. Exoperidial scales composed of interwoven hyphae, thick-walled and narrow lumen, some with yellowish content, 7.0–21 μm diam. Mesoperidial layer 380–670 μm wide, composed of hyaline hyphae.
with gelatinous walls and only the lumen is distinguishable, embedded in a gelatinous matrix; externally, some hyphae project from the gelatinous matrix and form spaced agglomerate with amorphous material, hyphae hyaline, 2.0–5.0 μm diam. Endoperidium composed of interwoven hyphae, slightly tick walled (walls up to 1.0 μm diam), clamped, 3.0–7.0 μm diam.

**Distribution:** American, following oak (**Quercus** L.) distribution.

**Comments:** *Calostoma lutescens* is characterized by the following features: basidiomata sulphur yellow, usually long-stalked; exoperidium dry to weakly gelatinous, warty, usually breaking down and forming a thorn ring at the base of the spore-sac; basidiospores globose ornamented with large round perforations (Castro-Mendoza et al. 1983). Size of basidiospores are reported to be up 10.5 μm in diam [6.0–8.0 μm in Coker & Couch (1928); (6.0–) 7.5–10.5 μm in Guzmán (1973); 5.5–8.0 μm in Castro-Mendoza (1983); 8.0–10 μm in Calonge et al. (2005a)] but the examined specimens (Costa Rican and Colombian) have basidiospores up to 14 μm (Fig. 2). The large-sized basidiospores led us think that the Central and South American specimens could represent a different taxon, but molecular evidences confirmed the species identification (Fig. 5). *Calostoma yunnanensis* L.J.Li & B.Liu, described from China, is a similar species with warty, pale yellow exoperidium, and globose basidiospores, 10.4–15.6 μm diam (Li et al. 1984).


**Phallaceae**


**Description:** see Dring (1980).

**Distribution:** pantropical and occasionally subtropical (Kasuya 2007).

**Comments:** the species is characterized by orange to reddish basidiomata, arms bifurcate or not and glebal mass spread in the central disc. American specimens not always tend to bifurcate arms. The large list of synonyms for this species (Dring 1980) is justified by the basidiomata polymorphy, which can vary in color (including a white form), size, arms and fertile disc morphology. *A. rubra* was several times reported from Costa Rica (Dring 1980, Saénz & Nassar 1982, Calonge et al. 2005a, Calonge & Mata 2006).

**Material examined:** COSTA RICA. PUNTARENAS: Monteverde Cloud Forest Reserve, L.D.Gómez & R.M.Alfaro 24856, VI/1986 (F 1074488).

**Clathrus columnatus** Bosc, Mag. Ges. Naturf. Freunde Berlin 5: 85 (1811)

**Description:** see Dring (1980).

**Distribution:** pantropical and subtropical (Dring 1980).

**Comments:** this species is characterized by 2-5 robust, spongy, orange columns free at the base and fused at the apex. The gleba is spread in the internal portion of the
Fig. 5. Single gene maximum likelihood analysis of the genus *Calostoma* using the ribosomal internally transcribed spacer sequences 1 through 2 (ITS; above phylogeny), and the ribosomal large subunit gene (LSU; below phylogeny). *Calostoma lutescens* from Costa Rica is indicated in bold font. Numbers adjacent to branches indicated bootstrap statistics ≥ 70%. The *C. miniata* branch of the LSU phylogeny is in gray because it has been shortened significantly to fit it into the figure.

538
columns and it is not confined to a glebifer. The species was previously reported from Costa Rica by Saénz & Nassar (1982) and Calonge et al. (2005a).

**Material examined:** COSTA RICA. ALAJUELAS, Benilda Sales, 4923, VII/1973 (F, Museo Nac. CR 58699).

*Laternea pusilla* Berk. & M.A.Curtis, J. Linn. Soc., Bot. 10(46): 343 (1868) [1869]

**Description:** see Sáenz (1976).

**Distribution:** neotropical (Calonge et al. 2005a).

**Comments:** *L. pusilla* is characterized by small sized-basidiomata (2.0–4.5 cm high), receptacle composed of 2–4, pale to bright red columns with a single glebifer situated below the columns junction. The most striking characteristic of this species is the presence of crests at the external portion of the columns. Although it is a species with neotropical distribution, it requires low temperature (16–20°C) to produce basidiomata (Sáenz 1976). Among the examined material, we could analyze the holotype (n° 45568) of *Colonnaria pereximia* L.D.Gómez and as stated by Dring (1980), the species is synonym with *L. pusilla*. *L. pusilla* was previously reported from Costa Rica by Saénz (1976), Saénz & Nassar (1982) and Calonge et al. (2005a).

**Material examined:** COSTA RICA. Fila de La Máquina, llano Bonito, Burger & Gómez, 3425, 20/VIII/1971 (F, Museo Nac. CR 45568, holotype of *C. pereximia*); camino de Vuelta de Jorco, Hammitt, Trejos & Gómez 49914, X/1972 (F, Museo Nac. CR 56496); Trinidad, Gómez 4021, XI/1973 (F, Museo Nac. CR 58648); PUNTARENAS: Monteverde Cloud Forest Reserve, Gómez & Alfaro 24881, VI/1986 (F 1110130).


**Description:** see Dring (1980).

**Distribution:** neotropical (Calonge et al. 2005a).

**Comments:** *L. triscapa* is characterized by 3–4, reddish to pinkish columns united at the tip where a glebifer bears the glebal mass. The species can be differentiated from *L. pusilla* since its basidiome is larger (up to 8 cm high) and columns are not externally crested. *L. triscapa* was previously recorded from Costa Rica by Saénz & Nassar (1982) and Calonge et al. (2005a).

**Material examined:** COSTA RICA. PUNTARENAS: Boruca, R.A.Ocampo 1000, VII/1975 (F, Museo Nac. CR 58496); ALAJUELAS, La Balsa de Atenas, J. & K.Utley 2598, 10/VII/1975 (F, Museo Nac. CR 57214).


**Description:** see Sáenz (1980).

**Distribution:** Costa Rica Sáenz (1980) and Mexico (Calonge et al. 2004).

**Comments:** basidiomata can be found on humus or rotten wood and are typically composed by four to five whitish arms fused at the apex. Glebal structure is compound and correspond to four or five (depending on the number of arms) glebiferous laminae which are attached to the inner upper surface of the arms. Different from other phalloid...
species, glebal mass is not foetid (Sáenz 1980, Pegler & Gomez 1994). Originally described from Costa Rica, later was also recorded from Mexico (Calonge et al. 2004).


**Description:** see Sáenz & Nassar (1982).

**Distribution:** tropical and subtropical (Kreisel 1996).

**Comments:** *P. indusiatus* may be characterized by reticulate receptaculum, presence of long indusium (reaching the base) and purplish pigments at the base of the volva and rhizomorphs. The indusium is usually white, but forms with different colors (yellowish and pinkish) may also be found (Kobayasi 1965, Guzmán et al. 1990). *

*P. indusiatus* was previously recorded from Costa Rica by Saénz & Nassar (1982) and Calonge et al. (2005a).

**Material examined:** COSTA RICA. ALAJUELAS, Finca Flor de Mayo en Rio Segundo de Alajuela, J. & K. Utley 3175, X/1975 (F, Museo Nac. CR 58891, 58892, 58893, 58894).

**Phallus duplicatus** Bosc, Mag. Gesell. naturf. Freunde, Berlin 5: 86 (1811)

**Description:** see Sáenz & Nassar (1982).

**Distribution:** America, Africa (Kreisel 1996).

**Comments:** This specie is similar to *P. indusiatus*, but it can be differentiated by its short, pinkish indusium with narrow meshes (1–2 mm) that extends about 3–5 cm below the cap (Coker & Couch 1928). Molecular studies on this species would be interesting to test if it is a good species or a morphological variation from *P. indusiatus*. *

*P. duplicatus* was previously recorded from Costa Rica by Saénz & Nassar (1982) and Calonge et al. (2005a).

**Material examined:** COSTA RICA. ALAJUELAS: Benilda Sales, VII/1973 (F, Museo Nac. CR 58684); GUANACASTE: R.A. Ocampo 392, VII/1973 (F, Museo Nac. CR 58689).

**Protophallaceae**

**Protubera jamaicensis** (Murrill) Zeller, Mycologia 40:644 (1948) Fig 3

Basidiome 1.9 cm high × 1.8 cm in diam, ovoid, gregarious on wood, attached to the substratum by a single, basal, hyphal strand, 0.8 cm in length; peridial surface greyish yellow (4C4), with some brownish patches, grooved, smooth; peridium very thin, less than 0.5 mm. Gleba greyish green (30D6) with whitish veins, waxy, probably gelatinous when fresh, arranged in lobes or irregular elongated plates that project nearly to the center of the basidiome, connected with the inner part of the peridium by sutures. Peridium composed of three distinct layers; first layer 25–180 μm wide, composed by hyphae embedded in a yellowish gelatinous matrix, hyphae 1.5–3.5 μm in diam, repent, loosely interwoven, simple-septate, thin or slightly thick-walled, hyaline, or 2.0–9.0 μm in diam, thick-walled, yellowish; second layer 80–180 μm wide, compact,
composed by inflated, slightly modified hyphae, 2.5–14 μm in diam, tightly interwoven, simple-septate, slightly thick-walled (walls up to 1.0 μm), brownish; third layer 55–450 μm wide, composed by hyphae embedded in a hyaline to pale green gelatinous matrix, hyphae clamped, hyphae with gelatinized walls and only lumen staining in phloxine, lumen 0.5–3.5 μm in diam, clamps 2.0–10 μm in diam. Gleba lobes are surrounded by the third layer and whitish veins that wrap the glebal chambers; veins widening near to the peridium and attached to the second peridial layer, sometimes observed between the second and the third peridial layer, composed of tightly interwoven hyphae, 4.0–7.0 μm in diam, without clamps, thick-walled, solid, hyaline. Basidia 8-spored; basidiospores ellipsoid, with truncate base, brownish, 3.0–4.0 × 1.5–2.0 μm, smooth.

**Distribution:** Jamaica (Murrill 1910, Coker & Couch 1928), Costa Rica (present study).

**Comments:** In the examined material, the basal hyphal strand does not extend into the center of the basidiome as showed in Coker & Couch (1928, pl. XV). However, it is possible that this structure was present in the fresh material and was not maintained in herbarium material, since it is very fragile. Microscopic characteristics of the peridium indicate that it was viscid when fresh, as described for *P. jamaicensis*. *Protuberana nipponica* Kobayasi seems to be morphological similar and a photo of this species presented by Imazeki et al. (1988) resemble the Costa Rican material. Imai & Kawamura (1958) described the outer layer of the peridium of *P. nipponica* as a compact tissue, composed by globose, oblong or cylindrical hyphal cells, which can reach up to 20 μm in diam. However, Malloch (1989) described it as a layer formed by densely interwoven hyphae. In both articles, there is no mention about *P. nipponica* having a viscid surface. Based on these arguments and geographical proximity, we conclude that the Costa Rican material corresponds to *P. jamaicensis*. This is the first record of this species from Costa Rica.

**Material examined:** COSTA RICA. CATARGO: Rio Estrella, L.D.Gómez 18331, VIII/1982 (F1100643).

*Protuberana maracuja* Möller, Bot. Mitt. Tropen 7: 10 (1895)  

**Fig. 4**

Basidiome 3.0 cm high × μm 3.2 cm in diam, subglobose, with apical dehiscence, gregarious on wood, attached to the substratum by few or several hyphal strands, up to 3.0 cm in length; peridial surface yellowish grey (3C2) to grey (3B1, 3C1), groovy, when fresh, then 0.4 cm high × μm 2.1 cm in diam, dorsiventrally compressed and very hard when dry, peridial surface yellowish brown (5D5). Gleba olive brown (4E4) when fresh, arranged in elongated elliptical plates which are immersed in a hyaline to whitish, translucent gelatinous matrix and radially arranged from the center, connected to the inner part of the peridium by sutures, compact when dry. Peridium composed of two layers; first layer 75–190 μm wide, compact, composed by pseudoparenchimatosus hyphae, globose, subglobose to elliptical, hyaline to yellowish, 9.0–49 μm in diam, with few layers of repent, hyaline hyphae above it; second layer 20–180 μm wide, compact, composed by hyaline hyphae, tightly interwoven, apparently simple-septate, slightly thick-walled, 2.0–4.0 μm in diam, where many large crystals forming rosette patterns are present. Gelatinous areas composed by hyaline hyphae embedded in a gelatinous matrix, apparently simple-septate, hyphae with gelatinized walls and only
lumen staining in phloxine, lumen 1.5–3.0 μm in diam. Basidia 8-spored; basidiospores ellipsoid, with truncate base, yellowish to brownish, 3.5–5.0 × 1.5–2.3 μm, smooth.

**Distribution:** Brazil (Möller 1985, Furtado & Dring 1967), Suriname (Fischer 1933), Jamaica (Dennis 1953) and Costa Rica (present study).

**Comments:** *P. maracuja* is separated from other species of the genus due to its pseudoparenchimatous peridial layer and presence of large crystals rosette arrangements (usually in the second peridial layer). Furtado & Dring (1967) described Brazilian material with 4-spored basidia, but our analysis agrees with Malloch (1989)’s opinion that the basidia are 8-spored. Furtado & Dring (1967) affirmed that Dennis (1953)’ report of *P. maracuja* from Jamaica actually corresponded to *P. jamaicensis*, but according to the article description, we think that Dennis’ identification might be accurate. As stated by Furtado & Dring (1967), records from Ceylon (Petch 1911, pl. IV), Sumatra (Boedijn 1932, pg. 75), and Pakistan (Ahmad 1952, pg. 5), do not seem to correspond to *P. maracuja*, which probably has a neotropical distribution. This is the first record of *P. maracuja* from Costa Rica.


**Acknowledgements**

We would like to thank Herbaria F, CORD and ICN for the access to the exsiccati; Esteban M. Crespo (Universidad Nacional de San Luis, Argentina) for the SEM photos; Clark L. Ovrebo (University of Central Oklahoma, U.S.A.) for sending useful literature; and Gabriel Grilli (Universidad Nacional de Córdoba, Argentina) for assistance in scanning photo slide. AWW would like to thank Dr. Andrew Minnen for *C. lutescens* collections from Maryland, USA. This study was financially supported by CAPES (Brazilian agency) and Red Latinoamericana de Botánica (RLB-Andrew W. Mellon Foundation Grant 2010-2011, RLB2011-P12).

**References**


Manuscript received June 29, 2012; accepted September 4, 2012.