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Contribution to the study of neotropical discomycetes: a new species of the genus *Geodina (Geodina salmonicolor* sp. nov.) from the Dominican Republic

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Abstract

Geodina salmonicolor sp. nov., a new neotropical / equatorial discomycetes of the genus *Geodina*, is here described and illustrated. The discovery of this new entity allowed us to propose another species of *Geodina*, until now a monospecific genus, and produce the first 28S rDNA genetic data, which supports this species is related to genus *Wynnea* in the *Sarcoscyphaceae*.

Key-words – 1 new species – *Ascomycota* – *Sarcoscyphaceae* – Sub-tropical zone Caribbeans – Taxonomy

Introduction

A study started more than 10 years ago in the area of Santo Domingo (Dominican Republic) by one of the authors allowed us to identify several interesting fungal species, both *Basidiomycota* and *Ascomycota*. Angelini & Medardi (2012) published a first report of ascomycetes in which 12 lignicolous species including discomycetes and pyrenomycetes were described and illustrated in detail, also delineating the physical and botanical characteristics of the research area.

During one of the forays in Santo Domingo, another unusual discomycetes in the shape of a small upturned bell was found, characterized by a faint pink to salmon-colored hymenium contrasting with the brownish external surface. A closer examination suggested that it was a species related to the genus *Geodina* Denison, but different from the only species so far described, *G. guanacastensis* Denison. The first collection consisted of only one apothecium found on a jar of the Jardín Botánico Nacional Dr. Rafael Ma. Moscoso of Santo Domingo. A few years later, a single isolated apothecium of the fungus was found again in two other different places.

We tried to study also the holotypus of *G. guanacastensis* located in CUP, in order to compare it with our findings and to have more familiarity with the phylogenetic position of the genus, but it was impossible because the Herbarium did not consider our request of loan.

Material & Methods

Fungal specimens were photographed in situ, using a digital camera Nikon Coolpix 8400, and then dried using an air dryer. Microscopic examinations and drawings from them were done

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employing dried specimens rehydrated in water, using an Optika optical microscope (BK 1301 model). Mounts were made in water. Melzer's reagent, floxin and cotton blue in lactic acid were used to highlight the spore ornamentation. The herbarium specimens selected for DNA extraction are listed in Table 1. Specimens collected in this study were deposited in JBSD herbarium (Jardín Botánico Nacional Dr. Rafael M. Moscoso, Dominican Republic).

Total DNA was extracted from dried specimens following a modified CTAB-based procedure (Murray & Thompson 1980). PCR amplifications were performed with the primers ITS1F-ITS4, ITS1F-ITS2, or ITS3-ITS4 (White et al. 1990, Gardes & Bruns 1993) to amplify the ITS1 and ITS2 rDNA regions, while LR0R and LR5 (Vilgalys & Hester 1990, Cubeta et al. 1991) were used to amplify the 28S rDNA region. Amplification protocol consisted in: 95°C initiation for 5 min; 35 cycles at 95°C for 45 s, 54°C for 30 s, and 72°C for 45 s; and a final step at 72°C for 10 min. PCR products were checked in 1% agarose gel prior to purification and sequencing with one or more PCR primers. Chromatograms were visually checked in MEGA5 (Tamura et al. 2011) to detect and fix ambiguous nucleotides and sequencing problems. Consensus sequences were submitted to GenBank (Table 1). Newly generated 28S rDNA sequences and their closest relatives in public databases (coming mainly from Pfister et al. 2008, Romero et al. 2012, Carbone et al. 2013, Hansen et al. 2013), were aligned in MEGA 5.0 software with its Clustal W application and then corrected manually. The final alignment of 28S rDNA sequences (including 304/762 variable sites) was loaded in PAUP* 4.0b10 (Swofford 2001) and subjected to MrModeltest 2.3 (Nylander 2004). Model GTR+I+G was selected and implemented in MrBayes 3.1 (Ronquist & Huelsenbeck 2003), where a Bayesian analysis was performed (two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 2.21M generations, standard deviation having fell below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis 2006) using the standard search algorithm (2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Table 1 28S rDNA sequences used in the phylogenetic analyses.

Species	28S rDNA	Species	28S rDNA	Species	28S rDNA
Chorioactis geaster	KC012672	*Phillipsia carnicolor*	JQ260812	*Sarcoscypha austriaca*	AY945856
Cookeina insititia	AY945861	*Phillipsia crispata*	JQ260813	*Sarcoscypha austriaca*	AY945855
Cookeina sp.	KY090805	*Phillipsia domingensis*	AY945844	*Sarcoscypha coccinea*	FJ176859
Cookeina sp.	KY090983	*Phillipsia domingensis*	JQ260817	*Sarcoscypha coccinea*	AY544647
Cookeina speciosa	AY945862	*Phillipsia lutea*	JQ260816	*Sarcoscypha occidentalis*	AY945846
Cookeina tricholoma	AY945860	*Phillipsia olivacea*	JQ260814	*Sarcoscypha sp.*	KC012700
Donadinia helvelloides	NG_042731	*Phillipsia olivacea*	JQ260815	*Sarcosoma globosum*	KC109215
Galiella rufa	FJ238401	*Phillipsia sp.*	KY498592	*Sarcosoma latahense*	FJ176860
Geodina salmonicolor	**MG597288**	*Phillipsia sp.*	KY498590	*Scutellinia scutellata*	DQ247806
Geodina salmonicolor	**MG597287**	*Phillipsia subpurpurea*	KY498591	*Trichaleurina celebica*	KF418258
Kompsoscypha phyllogena	JQ260810	*Pithya cupressina*	JQ260818	*Trichaleurina javanica*	JX669861
Microstoma floccosum	JN012013	*Plectania melastoma*	JX669850	*Urnula craterium*	AY945851
Microstoma floccosum	DQ220370	*Pseudopithyella minuscula*	AY544658	*Wolfina aurantiopsis*	AY945859
Nanoscypha tetraspora	DQ220374	*Pseudoplectania nigrella*	NG_042728	*Wynnea americana*	AY945848
Phillipsia carnicolor	JQ260811	*Rickiella edulis*	JQ260809	*Wynnea sparassoides*	EU360917

Results

Analysis of 28S rDNA suggested that the samples collected are significantly related to genus *Wynnea* Berk. & M.A. Curtis and subfamily Wynneae of the *Sarcoscyphaceae* Le Gal ex Eckblad (Fig. 1). A significant support for subfamily *Boedijnopezizeae* was also obtained, but not for *Sarcoscypheae* Fr., in contrast with previous authors (Romero et al. 2012). No intraspecific variability of 28S or ITS rDNA sequences was observed between the samples analyzed, suggesting that they belong to a single taxon, which is here proposed as new species of *Geodina* because of the morphological similarity (but not identity) with the type species of this genus, *G. guanacastensis*.

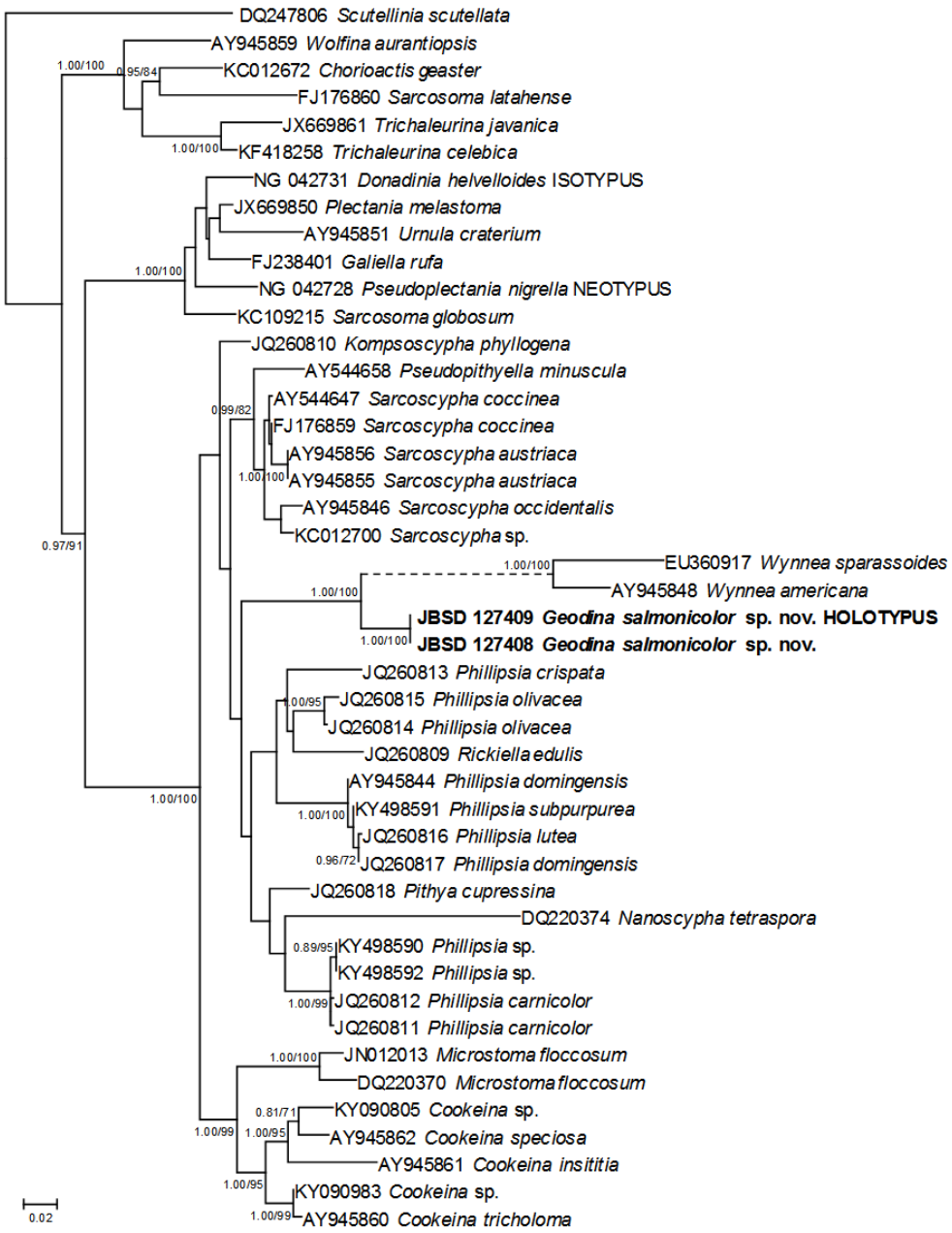

Figure 1 – 50% majority rule consensus 28S rDNA phylogram of the family Sarcoscyphaceae obtained in MrBayes from 16 575 sampled trees. Nodes supported by >0.95 Bayesian PP or >70% ML BP are shown annotated. Sequences obtained in the present study are shown in bold characters.

Geodina salmonicolor Angelini & Medardi, sp. nov. Figs 2–4

MycoBank: MB823851

Etymology – for the hymenial color, delicately rosy-orange or pale pink-orange.

Diagnosis

Fruitbody funnel-shaped, up to 40 mm height and 25–30 mm diam. Apothecium cup-shaped to urceolate. Hymenial surface smooth, faint pink salmon to pale pink-orange. Receptacle surface pale chocolate-brown, pale brown-greyish, with dark brown hairs, zoned by some rings alternately pale and dark, more conspicuous near the margin. Margin entire or shortly cracked. Stalk up to 25 mm long, tapered and curved, smooth, dark brown. Flesh waxy but elastic, whitish or slightly pink in the cup, brownish and fibrous in the stalk.

Ascospores 35–39 × 14–15 µm, ellipsoid or citriform, smooth and oil-dropped when young, hyaline, after that developing ornaments consisting of coarse, more or less irregularly arranged ridges ribs up to 2 µm broad and high, curved, often anastomosed to form portions of a partial reticulum. Asci 300–380 (450) × 18–21 µm, 8-spored, with long, curved and gradually tapered base, unamyloid, sub-operculates. Paraphyses cylindrical at the base (2–3 µm), broadening up to 5 µm at the top and near the septa, scarcely anastomosed only in the lower part. Subhymenium not observed. Medullar excipulum up to 600 µm thick; textura intricata, hyphae 2.5–3 µm, interwoven, branched, septate, hyaline. Ectal excipulum 350–400 µm thick; textura globulosa or globulosa-angularis, cells up to 25 µm diam., 25 × 10 µm or 20 × 15 µm if compressed, dark brown to blackish-brown. Hairs 500–550 µm long, 10–-15 µm large at the top and 20–40 µm at the base, made up of fasciculate, cylindrical, closed hyphae 4–4.5 µm, with a few septa, dark brown.

Holotypus hic designatus: Dominican Republic, Santo Domingo, on humous soil and litter in the natural part of the Jardín Botánico Nacional dr. Rafael Ma. Moscoso, under tropical mixed indigenous hardwood trees, 08 Nov 2011; leg. C. Angelini, det. C. Angelini & G. Medardi (JBSD127409, GenBank acc. n. MG597290, MG597288).

Extended description

Fruitbody funnel-shaped, up to 40 mm high and 25–30 mm diam. Apothecium like an overturned bell, strongly cup-shaped (up to 20 mm deep). Hymenial surface smooth, delicate pink salmon to pale pink-orange. Receptacle surface more or less pale chocolate-brown, tobacco with greyish reflexes, with clearly visible dark brown hairs up to the edge; some pale and dark concentric rings can be seen, especially near the margin. Margin entire or shortly cracked, undulated, slightly lobed, at times somewhat reflexed. Stalk up to 25 mm long, cylindrical, often curved, tapered toward the bottom (1–2 mm diam.) and wider at the top where it reaches the cup, 1.5–4.5 mm diam., smooth, dark brown, darker at the base; some longitudinal furrows and/or small cavities can be present near the junction of the stalk with the apothecium. Flesh waxy but not brittle, rather elastic, whitish or faintly pink in the apothecium, brownish and more fibrous in the stalk.

Ascospores 35–39 × 14–15 µm, elliptical or citriform, heavily sculptured, thick-walled (up to 2–2.5 µm), when young smooth and with some oil drops, hyaline, uniseriate in the asci; spore ornaments consisting in coarse, more or less longitudinal, oblique or transversal ridges up to 2 µm broad and high, curved, often anastomosed and joined to form portions of irregular or partial reticulum. Asci 300–380 (–450) × 18–21 µm, 8-spored, cylindrical, thick-walled (up to 3 µm), with long, often curved and gradually tapering base, unamyloid, sub-operculates; operculum typically eccentric and quite small (5–6 µm), round or elliptical. Paraphyses cylindrical at the base (2–3 µm diam.), clavate and enlarged up to 5 µm at the top and near the septa, some of them forked, sometimes anastomosed at the base, without oil-drops. Subhymenium not observed. Medullary excipulum up to 600 µm thick; *textura intricata*, hyphae 2.5–3 µm broad, interwoven, forked, septate, hyaline. Ectal excipulum 350–400 µm thick; *textura globulosa* or *globulosa-angularis*, cells up to 25 µm diam., globose or compressed (and then of 25 × 10 µm or 20 × 15 µm), wall-thickened (1–1.5 µm), more or less dark brown to blackish-brown. Extracellular dark brown pigments present. Hairs 500–550 µm long, 10–15 µm large at the top and 20-40 µm near the base,

originating from the ectal excipulum, single or clustered, composed of 2-3 or several fasciculate hyphae, more or less parallel and densely packed. Hyphae are 4–4.5 µm broad, cylindrical, with rounded apices, thin-walled (0.5–1 µm), with a few septa, dark brown; they are more abundant and shorter at the base, making every single hair of a strictly triangular shape. Some scattered, small scales (approximately up to 90 × 45 µm) can be found among the hairs, triangular, made up of hyphae similar to those composing the hairs but markedly shorter, septate (maybe hairs still developing).

Habitat – generally solitary on rich soil and litter in deciduous tropical and equatorial woods, November–December. As stated by DENISON (1965: 651) for *G. guanacastensis,* the fungus grows on soil, but the stalk could reach some buried wood below.

Figure 2 – *Geodina salmonicolor*. A, B first collection (JBSD 127409, holotypus). C, D second collection (ANGE 759). E, F third collection (JBSD 127408).

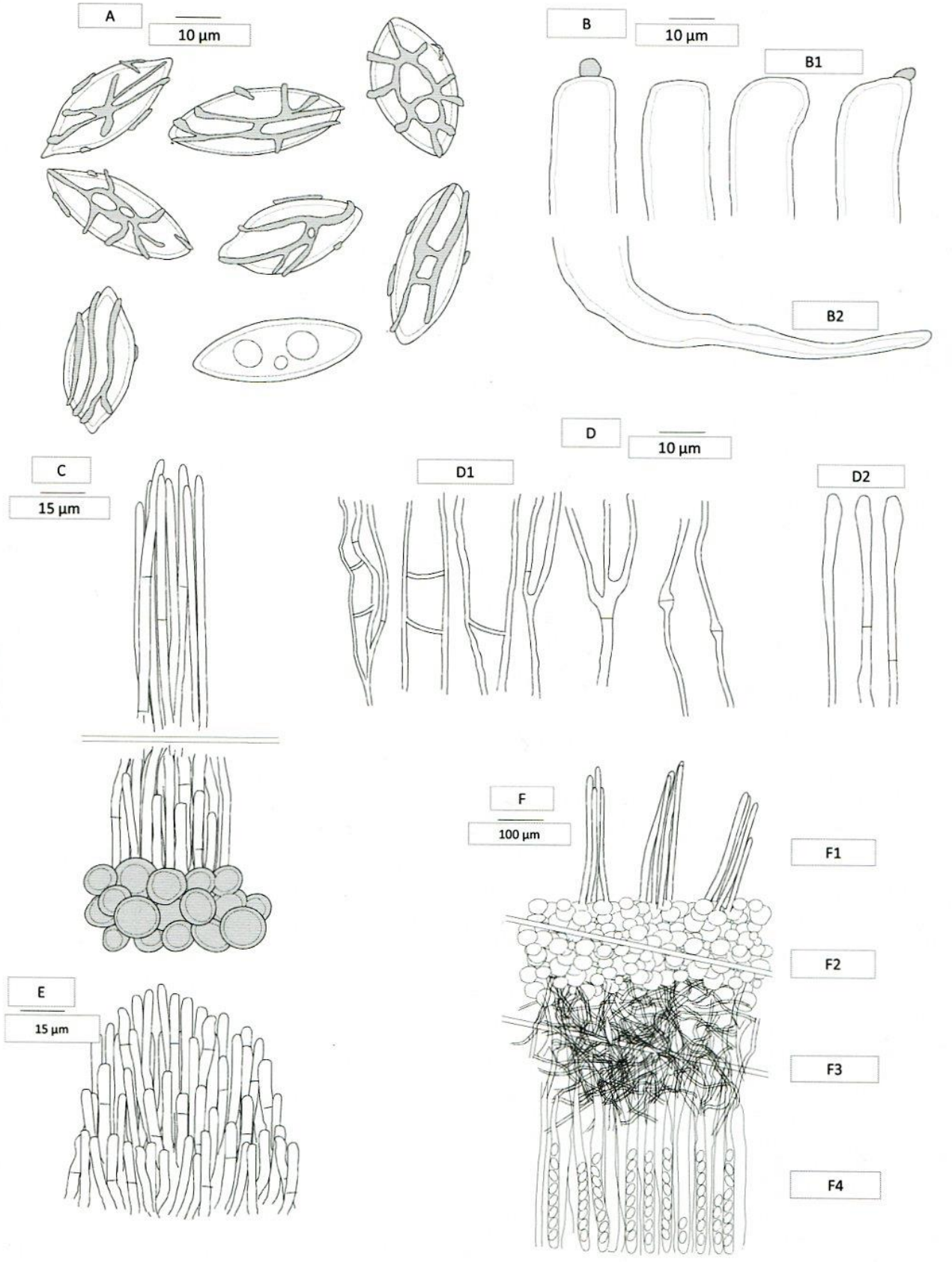

Figure 3 – *Geodina salmonicolor*. A Released ascospores. B Asci (B1- apex shape. B2 base shape). C Hairs. D Paraphyses (D1 lower part. D2 upper part). E Scales near the hairs base. F Diagrammatic vertical section of one apothecium (F1 Hairs. F2 Ectal excipulum F3. Medullar excipulum. F4 Hymenium).

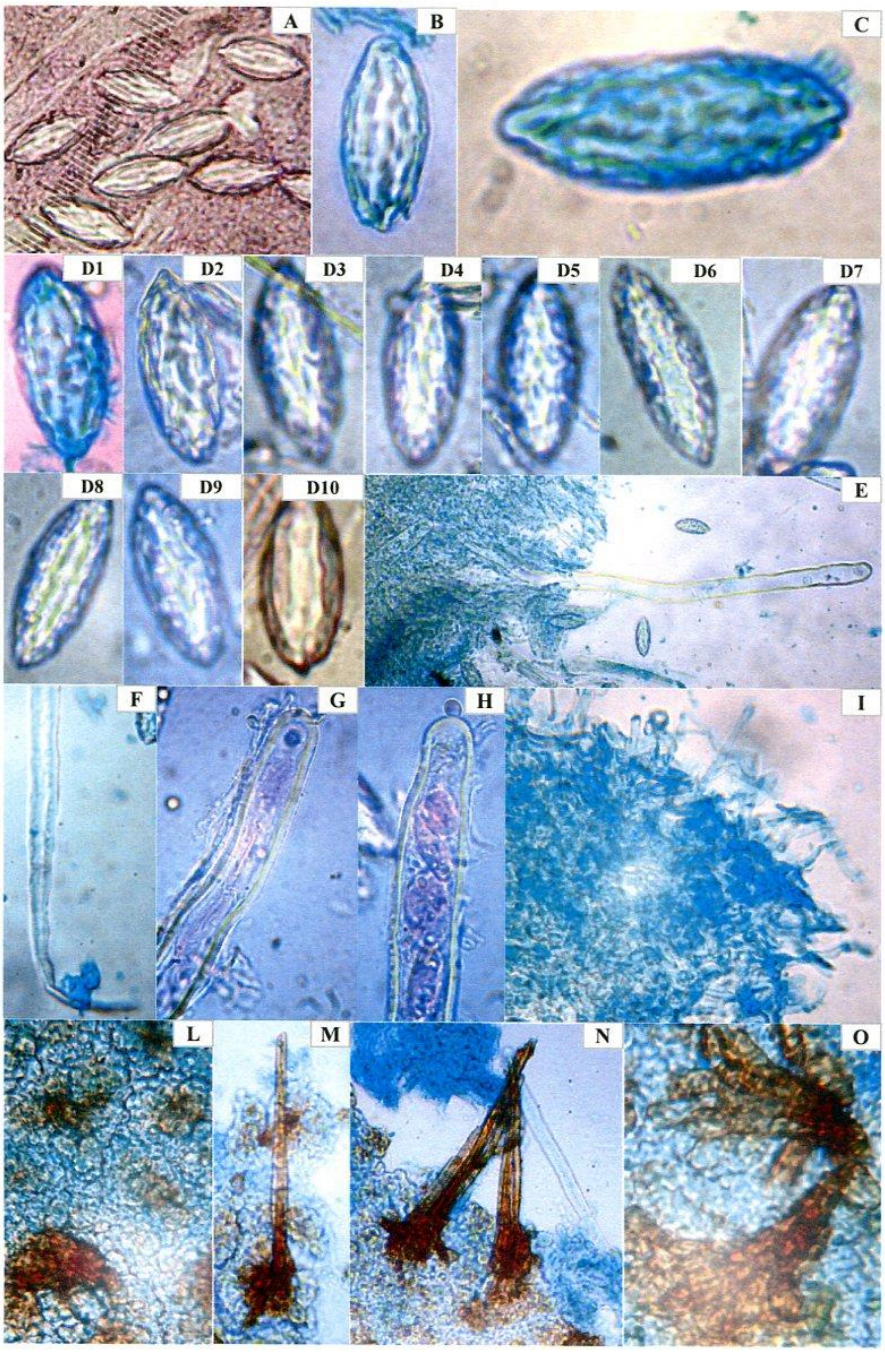

Figure 4 – *Geodina salmonicolor* - A Ascospores. B, C Ascospores. D1÷D10 Appearance and general outline of the spore surface ribs. E Ascus in Lactic blue. F Ascus base. G Ascus top. H Ascus top and operculum. I Medullar excipulum. L Ectal excipulum. M, N hairs. O scales. (A, D2÷D10, G, H – Phloxin; B, C, D1, E, F, I, L, M, N, O - Lactic blue). [Scale bar: — (5 mm): A, G, H = 20 µm; B, D1÷D10 = 10 µm; C = 6 µm; E = 50 µm; F = 25 µm; I = 100 µm; L, M, N, O = 80 µm].

Other specimens examined – 23 Nov 2016, Sosúa (P.to Plata) loc. Cemetery, leg. C. Angelini, det. C. Angelini & G. Medardi (ANGE759); 25 Dec 2016, Sosúa (P.to Plata) loc. Cemetery, leg. C. Angelini, det. C. Angelini & G. Medardi (JBSD127408).

Discussion

The monotypic genus *Geodina* belongs to the family *Sarcoscyphaceae* (Wijayawardene et al. 2017, 2018), integrated by stalked species with tough or elastic apothecia, commonly growing on

logs or wood debris in early stages of decay. Their elastic consistency is due to the *textura intricata* (prosenchyma) generally present in large amount in the structure of the flesh. Asci of the *Sarcoscyphaceae* are sub-operculate, markedly thick-walled (mainly at the top) and typically with an eccentric operculum. Eckblad (1968) distinguished two tribes in that family, *Sarcoscypheae* Fr. and *Urnuleae* Eckblad, the former based on light colour and presence of carotenoid pigments, the latter with dark tints and lacking of carotenoid. He classified *Geodina* within *Sarcoscyphae* because of its morphological features.

Morphologically, *Geodina* recalls *Cookeina* Kunze; because of their sub-operculate asci, hairs shape and structure, and the presence of prosenchymatous tissue in the structure of the trama. However, *Cookeina* has the base of the asci abruptly tapered and ending in a thin, often curved thread-like appendage, the paraphyses are closely anastomosed at all levels by transversal or oblique septa, and the ascospores show only some longitudinal thin lines (Angelini & Medardi 2012, Dennis 1970, Le Gal 1953, Seaver 1928). According to Denison (1965) there is another important difference between the two genera: all asci mature simultaneously in *Cookeina,* but in separate groups in *Geodina*, so that in the same section we can observe asci at different stages of development, with ascospores completely mature or empty.

Denison (1965) created the genus *Geodina* for the only species known at that time, *G. guanacastensis*, characterized by its stalked apothecia with an hymenial surface pale orange to light yellow-orange and a concolor outer surface looking darker because of a coat of dark brown hairs 300–800 µm long, smaller ascospores 22–25 × 11–13 µm, and fruiting on naked soil. *Geodina salmonicolor* differs because of 1) brownish zoned outer surface, 2) larger spores, 3) presence of some septa in the thin-walled hyphae composing the hairs, 4) shorter hairs mixed with scattered scales, 5) a smaller and more regular operculum than that drawn by Denison (l.c.: 652), 6) oil drops visible only in young ascospores, and 7) ectal excipulum of *textura globulosa* or *globulosa-angularis* instead of *prismatica* or *angularis*.

Short dichotomic key for the species of the genus *Geodina*

1 – Ascospores 22–25 × 11–13 µm. Hymenial surface pale orange to light yellow-orange; outer surface concolorous. Hairs 300-800 µm long, made up by hyphae without septa
G. guanacastensis

1 – Ascospores 35–39 × 14–15 µm. Hymenial surface delicate pink salmon to pale pink-orange; outer surface more or less pale chocolate-brown, tobacco with greyish reflexes, zoned by some rings alternately pale and dark, more closed near the margin. Hairs 500-550 µm long, made up by septate hyphae ***G. salmonicolor***

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