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A new species of *Scytinopogon* from the island of Príncipe, Republic of São Tomé and Príncipe, West Africa

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Abstract

Scytinopogon havencampii is described as new from material collected from a non-ectotrophic forest on the West African island of Príncipe. Diagnostic features include brown, non-flattened branches with white tips, small, white, ellipsoid, coarsely echinate basidiospores, 2-spored basidia, non-inflated hyphae, and brittle basidiomes that dry grayish white. Phylogenetic analyses of nLSU sequence data confirm placement of this new taxon within the Trechisporales. A comprehensive description, photograph of basidiome, SEM of basidiospores, DNA sequences, and comparisons with phenetically similar taxa are provided.

Key words – coral fungi – Gulf of Guinea – systematics – taxonomy – Trechisporales

Introduction

The west African islands of São Tomé and Príncipe, lying along the magmatic Cameroon Volcanic Line (aka Guinea Line) in the Gulf of Guinea, are oceanic islands in the sense of Carlquist (1965), arising from a volcanic plume and never connected to the African continent. São Tomé at 13+ my and Príncipe at 31+ my (Lee et al. 1994) are biodiversity hotspots with high levels of endemism (MacArthur & Wilson 1967); in 2013, the island of Príncipe was designated a UNESCO World Biosphere Reserve. In April 2006 (2 wk) and April 2008 (3 wk), expeditions led by scientists from the California Academy of Sciences and joined by mycologists from San Francisco State University visited São Tomé and Príncipe to document the diversity of plants, amphibians, marine invertebrates and macrofungi. A first account of the macrofungi collected during these expeditions was the publication of a new stinkhorn, *Phallus drewesii* Desjardin & B.A. Perry, from São Tomé (Desjardin & Perry 2009). We continue reporting interesting species from the island nation with the description of a new coralloid homobasidiomycete from the oldest of the islands, Príncipe.

The earliest reports of fleshy macrofungi from São Tomé and Príncipe were by Bresadola and Roumeguère (1890), describing collections made by Moller, Quintas and Newton in 1885. Although the majority of species they reported were polypores, a few agarics, gasteromycetes, stereoid fungi, jelly fungi, ascomycetes and myxomycetes were included. Pertinent to this paper, was the description of three new coralloid homobasidiomycetes, viz., *Clavaria henriquesii* Bres. &

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Roum., *Lachnocladium mollerianum* Bres. & Roum., and *Pterula subaquatica* Bres. & Roum. Since then, African coralloid fungi have received little attention. Corner (1950, 1966, 1970) and others (Roberts 1999, Douanla-Meli 2007) reported a number of species from mainland Africa, but the only taxa reported from São Tomé and Príncipe are the former three species, treated by Corner (1950) as *Ramaria henriquesii* (Bres. & Roum.) Corner, *Ramaria molleriana* (Bres. & Roum.) Corner, and *Pterulicium xylogenum* (Berk. & Broome) Corner, respectively. Among the interesting species collected from a non-ectotrophic forest on Príncipe in 2008, was a brown, white-tipped *Scytinopogon* with small, coarsely echinate spores formed on 2-spored basidia, described as new herein.

Materials & Methods

Specimens were dried on a Nesco food dehydrator, packed in airtight plastic bags and hand carried back to the US. Macromorphological data were derived from fresh specimens, whereas micromorphological data were derived from dried specimens rehydrated in ethanol followed by distilled water, 3% KOH or Melzer's reagent. Color terms and notations are those of Kornerup and Wanscher (1978). Spore statistics include x_m, the arithmetic mean of the spore length by spore width (± standard deviation); Q, the quotient of spore length by spore width in any one spore, indicated as a range in variation in n spores measured; Q_m, the mean of Q-values (± standard deviation). For the micrographs of basidiospores, a small portion of dried hymenial tissue was mounted on an aluminum stub using graphite paint, coated with 8 nm of iridium in a Cressington 208HR sputter coater, and examined at an accelerating voltage of 2 keV in a Carl Zeiss Ultra 55 field emission scanning electron microscope (FE-SEM).

Total genomic DNA was extracted from dried material using the E.Z.N.A. Forensic DNA Kit (Omega Bio-Tek, Inc., Norcross, GA) following the manufacturer's instructions. PCR and cycle sequencing protocols followed those outlined in Perry et al (2007). The nuclear ribosomal large subunit (nLSU) and internal transcribed spacer region (ITS) were symmetrically amplified using primers LROR/LR7 (Moncalvo et al. 2000) and ITS1-F/ITS4 (Gardes and Bruns 1993; White et al. 1990), respectively. Amplification products were cleaned using the Exo-SAPit kit (Affymetrix, Santa Clara, CA), and sent to ELIM Biopharmaceuticals (Hayward, CA) for sequencing. Resulting sequence fragments were edited and assembled in Geneious 8 (Biomatters Limited, Auckland, New Zealand). ClustalW (Larkin et al. 2007) and Mesquite (Maddison & Maddison 2015) were used to align the nLSU sequence of *Scytinopogon havencampii* within a broader sampling of the Trechisporales as sampled in Birkebak et al. (2013) and Telleria et al. (2013). The nLSU and ITS sequences of *Scytinopogon havencampii* have been deposited in GenBank (nLSU–KT253947, ITS–KT253946), and the aligned nLSU dataset and associated tree file have been deposited in TreeBASE (submission #17611).

Maximum likelihood analyses were conducted in RAxML 8.1.11 (Stamatakis 2014) under the GTRCAT model using the default parameters and run on the CIPRES Science Gateway (Miller et al. 2010), with node support assessed by 1000 RAxML bootstrap (BS) replicates. Bayesian analyses were performed using Metropolis Coupled MCMC methods as implemented in MrBayes 3.2.3 (Ronquist et al. 2012) under a GTR+I+G model of sequence evolution as determined under the Bayesian Information Criterion in jModelTest 2.1.6 (Darriba et al. 2012, Guindon & Gascuel 2003). Bayesian analyses consisted of two parallel searches, run for 8 million generations on the CIPRES Science Gateway, and initiated with random starting trees. Six chains were sampled every 800 generations for a total of 10,000 trees each, sampled from the posterior distribution. Those topologies sampled prior the runs reaching a split deviation frequency of 0.01 were discarded, while the remaining were used to calculate the posterior probabilities of the individual clades. The default settings were used in MrBayes to set unconstrained branch lengths and uninformative topology priors.

Based upon the maximum likelihood results, constrained topology analyses were conducted to evaluate the significance of alternative tree topologies in which the genus *Trechispora* was constrained to monophyly. A constrained topology was manually specified in GARLI 0.951(Zwickl

2006) and a tree search was performed under a GTR+I+G model of sequence evolution with all parameters estimated by the program. The resulting topology was compared with the most likely, unconstrained topology using the Shimodaira & Hasegawa (1999) test as implemented in PAUP* (Swofford 2003), with the resampling estimated log-likelihood (RELL) method and 1K BS replicates.

Taxonomy

Scytinopogon havencampii Desjardin & B.A. Perry, **sp. nov.** Figs. 1–2

Mycobank number: MB 813054

Facesoffungi number: FoF 00891

Diagnosis – Basidiome coralloid, 90–100 mm tall × 60–70 mm broad. Stipe 20–25 × 5–6 mm, confluent, white to pale orangish white. Branches 3–4 mm diam, round in cross-section, pale brownish orange at the base grading upwards to 1–2 mm diam, grayish brown to brown with white acute tips; tissues unchanging where cut or bruised; texture rather brittle. Basidiospores 5.2–6.5 (–7) × 3.5–4.2 µm, ellipsoid, coarsely echinate, spines 0.5–1 mm tall, hyaline in 3% KOH, presumably white in deposit. Basidia primarily 2-spored, clamped. Hymenial cystidia absent. Hyphae 3–8 µm diam, thin-walled, clamped but not at every septum. Scattered in soil in secondary forest of non-ectotrophic trees. Holotype: Africa, Príncipe, Roça Pico Papagaio, 21 April 2008, D.E. Desjardin DED8300 (SFSU).

Etymology – Named in honor of several generous gentlemen from Haven Camp, California, who graciously provided partial support for this research.

Fig. 1 – Basidiome of *Scytinopogon havencampii* (DED8300). Natural size.

Basidiome coralloid, 90–100 mm tall × 60–80 mm broad, moderately open, densely branched. Stipe 20–25 × 5–6 mm, confluent, solid, white to pale orangish white (5A2). Primary lower branches 3–4 mm diam, pale brownish orange (6C3) to dingy tan; secondary branches 2–3 mm diam, grayish brown (7D3), axils V-shaped; tertiary and terminal branches 1–2 mm diam, dichotomous or rarely trichotomously branched, axils V-shaped, brown (7E3) with a hint of violet; tips acute, white. All tissue unchanging where cut or bruised; texture rather brittle. Odor not distinctive. Dried grayish white.

Basidiospores 5.2–6.5 (–7) × 3.5–4.2 µm [x_m = 6.1 ± 0.4 × 3.8 ± 0.2 µm, Q = 1.5–1.7, Q_m = 1.6 ± 0.04, n = 25, s = 1], ellipsoid, coarsely echinate, spines 0.5–1 mm tall, conical, hyaline in 3% KOH, presumably white in deposit. Basidia primarily 2-spored, rarely 3- or 4-spored, 19–23 × 5.7–8.5 µm with sterigmata 5–9.5 µm long, clavate, hyaline, not golden-refringent, clamped. Hyphae 3–8 µm diam, cylindrical, hyaline, smooth, thin-walled, clamped but not at every septum.

Habitat and known distribution – Scattered in soil in secondary forest of non-ectotrophic trees. Príncipe, Africa.

Material examined – Africa, Príncipe island, Roça Pico Papagaio, N01˚37.182', E07˚23.474', 21 April 2008, collected by D.E. Desjardin, DED8300 (Holotype – SFSU).

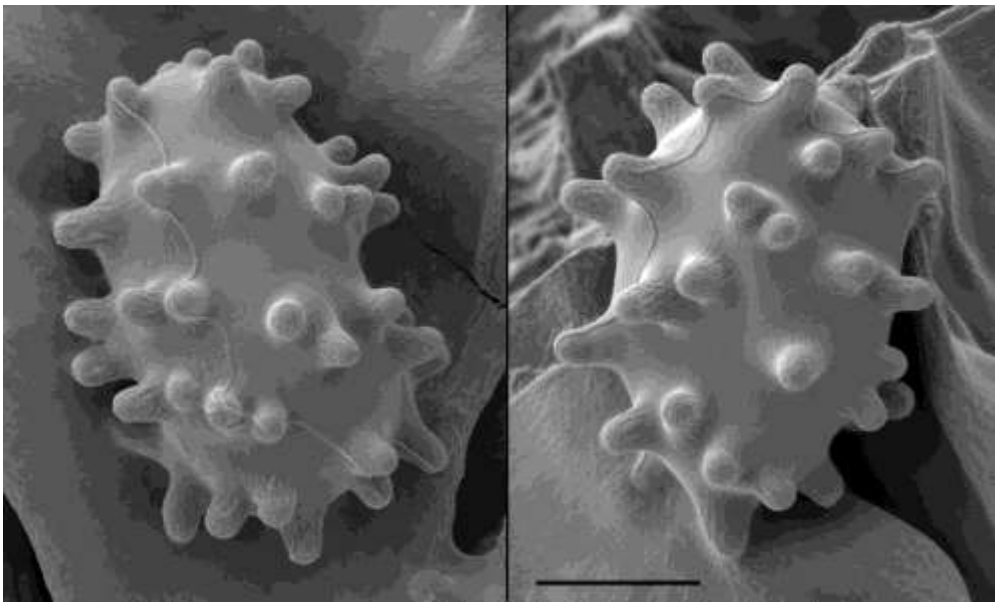

Fig. 2 – Scanning electron micrographs of basidiospores of *Scytinopogon havencampii* (DED8300). Scale bar = 2 µm.

Notes – Distinctive features of *Scytinopogon havencampii* include relatively small, open, densely branched, brittle basidiomes with brown, white-tipped branches arising from a white to pale orangish white stipe, branches that are not flattened, tissues that do not change color when bruised, small, white, coarsely echinate, ellipsoid (not angular) basidiospores with mean 6.1 × 3.8 µm, short, primarily 2-spored basidia, clamped, non-inflated hyphae 3–8 µm diam, and growth in soil in non-ectotrophic secondary forest on the oceanic island of Príncipe in the Gulf of Guinea,West Africa.

After the exclusion of ambiguously aligned and poorly represented regions, the nLSU data set consists of 830 nucleotides for 27 ingroup taxa and contains 313 variable positions. Three species of *Sistotrema* and two species of *Repetobasidium* (Polyporales) were used as outgroup taxa for rooting purposes. Maximum likelihood analyses produced a single topology (Fig. 3). Bayesian analyses reached a split deviation frequency below 0.01 after 1.5 million generations, and the first 1875 trees were excluded as the burn-in. *Scytinopogon havencampii* is resolved as sister to *Scytinopogon angulisporus* with 92% maximum likelihood BS and 1.0 Bayesian posterior probability (PP) support. Both species of *Scytinopogon* sampled are embedded within the genus *Trechispora*, but with limited support values (80% BS and <0.70 PP). Other genera of Trechisporales represented by more than a single taxon, including *Brevicellicium*, *Subulicystidium*, *Sistotremastrum* and *Tubulicium* are all well-supported as monophyletic (>95% BS and 1.0 PP). Constrained topology analyses (data not shown) fail to recover any trees that are significantly more likely than the unconstrained topology. Comparison of the *S. havencampii* ITS sequence using BLAST indicates highest overall identity (86%) to three undetermined species of *Scytinopogon* currently represented in GenBank.

Discussion

Most known *Scytinopogon* species form white to cream basidiomes with flattened branches, and a rather tough texture. Only two species are known to form pigmented basidiomes. *Scytinopogon havencampii* shows strongest phenetic similarity to *S. robustus* (Rick) Corner, a species described from Brazil and subsequently reported from Puerto Rico, but the latter has pale grey to grayish violet, flattened branches with white subulate to palmate tips, angular-ellipsoid basidiospores, 4-spored basidia, and hyphae up to 25 µm diam that are constricted at the septa (Corner 1970). Another pigmented species, *Scytinopogon echinosporus* (Berk. & Broome) Corner, known from Sri Lanka and Java, differs in pale purple or lilac, often flattened branches with dark violet-brown tips, ellipsoid to angular basidiospores, 4-spored basidia, inflated hyphae (up to 12 µm diam), and basidiomes that dry black.

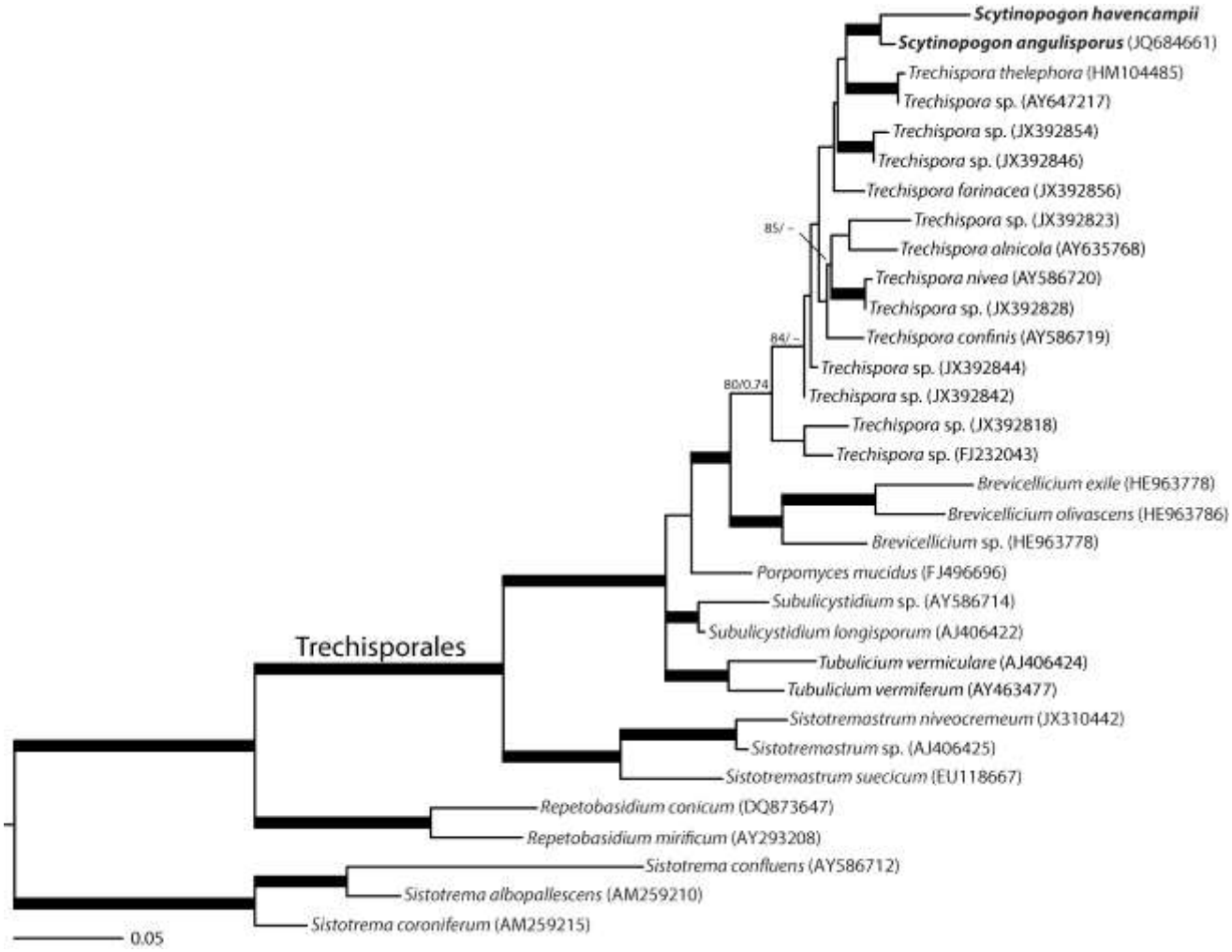

Fig. 3 – Maximum likelihood phylogeny of Trechisporales based on nLSU sequence data. *Scytinopogon havencampii* and *S. angulisporus* are indicated in bold type. Values separated by / refer to nonparametric ML bootstrap proportions and Bayesian posterior probabilities. Only values greater than 70/0.70 are shown (- designates a value below 70% or 0.70). Nodes receiving support values greater than 90/0.95 are highlighted in bold.

In a remarkable example of convergence, the new *Scytinopogon* species is reminiscent of several paleotropical *Ramaria* taxa, although the latter form ferruginous or ochraceous basidiospores with different ornamentation. *Ramaria zippelii* (Lév.) Corner, described from material collected in Java (Léveillé 1844) and subsequently reported throughout Indomalaysia (Corner 1950) differs in forming alutaceous, fuliginous-tan or dirty ochraceous basidiomes that

rapidly turn purplish vinaceous where bruised, usually have a strong paracresol odor, and form larger basidiospores (10–15 × 5–8 µm) on 2-spored basidia. Corner (1950) described *R. zippellii* var. *gracilis* Corner from material collected in Johore, Malaysia, distinguishing it from the type variety because of smaller and more slender basidiomes, smaller basidiospores 7–10 × 4.5–6.5 µm, and basidia that are mostly 3- or 4-spored. Corner (1950) transferred several similar African coralloid species into *Ramaria*, but with the caveat that they should be compared to *Scytinopogon*. *Ramaria ochracea* (Bres.) Corner (type *Lachnocladium ochraceum* from the DR Congo) forms basidiomes that dry fuscous, lack a stipe, have branches that are ochraceous-pulverulent from basidiospores, and grows on rotten tree trunks (Corner 1950). *Ramaria durbana* (Van der Byl) Corner (type *Clavaria durbana* from South Africa) forms smaller (–25 × 15 mm), flesh-colored basidiomes, pale ochraceous basidiospores, and grows on wood. The latter species is reported as a synonym of *Scytinopogon pallescens* in Species Fungorum and MycoBank.

Phylogenetic placement. Jülich (1981) was the first to indicate a relationship between the coralloid *Scytinopogon*, stipitate-hydnoid *Hydnodon* and resupinate *Trechispora*, accepting *Scytinopogon* in its own family, Scytinopoganaceae Jülich, *Hydnodon* and *Trechispora* in the Hydnodontaceae Jülich, and together placing them in the order Hydnodontales Jülich. Based on similar micromorphology, Ryvarden (2002) transferred *Hydnodon thelephorus* (Lév.) Banker into *Trechispora*. Molecular data support numerous *Trechispora* species (Larsson et al. 2004, Telleria et al. 2013), the monotypic *Hydnodon* (Larsson et al. 2011), the genera *Brevicellicium*, *Subulicystidium*, *Porpomyces*, *Sistotremastrum* (Larsson 2007, Telleria et al. 2013), *Tubulicium* and *Scytinopogon angulisporus* (Pat.) Corner (Birkebak et al. 2013) in a monophyletic lineage dubbed the trechisporoid clade, sister to the Polyporales. The ordinal name Trechisporales K.H. Larsson (in Hibbett et al. 2007) was established for the trechisporoid clade, even though the earlier valid and legitimate Hydnodontales was available (ICN Art. 11.10 – "the principle of priority does not apply above the rank of family"). Birkebak et al. (2013) reported that nLSU sequences of *Scytinopogon angulisporus*, a species described from Venezuela, clustered within the genus *Trechispora*. Our results agree with recent molecular analyses of Trechisporales (Birkebak et al. 2013; Telleria et al. 2013) and further suggest a close, yet poorly resolved, phylogenetic placement of *Scytinopogon* within the genus *Trechispora*. Constrained analyses forcing both sampled species of *Scytinopogon* out of a monophyletic *Trechispora* fail to recover significantly improved tree topologies, suggesting uncertain placement of *Scytinopogon* based on the current sampling. The type species of *Scytinopogon*, *S. pallescens* (Bres.) Singer, described from the DR Congo, has not been sequenced, but has been recognized as a synonym of *S. angulisporus* (described from Venezuela) by Corner (1950) and Petersen (1988). Species Fungorum reports *S. pallescens* as a distinct species. The type species of *Trechispora*, *T. onusta* P. Karst (type locality Finland), has apparently not been sequenced and is not included in any published phylogenies. The coralloid genus *Scytinopogon* should not be accepted as a synonym of the resupinate *Trechispora* until the type species of both genera are sequenced and compared.

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