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Author(s): Michaela Schmull, Manuela Dal-Forno, Robert Lücking, Shugeng Cao, Jon Clardy, and James D. Lawrey
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Dictyonema huaorani (Agaricales: Hygrophoraceae), a new lichenized basidiomycete from Amazonian Ecuador with presumed hallucinogenic properties

Michaela Schmull1, Manuela Dal-Forno2, Robert Lücking3, Shugeng Cao4, Jon Clardy5 and James D. Lawrey6,7

Abstract. Dictyonema huaorani, a new species represented by a well-developed specimen found in the Ecuadorian Amazon region, is described in this paper. The material was collected during a Harvard ethnobotanical expedition in 1981 and originally determined by Mason E. Hale Jr. as belonging in the genus Dictyonema (D. sericeum s.lat.) and possibly representing an undescribed species. The species is morphologically distinctive in forming densely woven, semicircular thalli, closely resembling those of the paleotropical D. ligulatum but lacking clamps and with hyphal sheath around the photobiont filaments that resembles those of Cyphellostereum species. The species was reported to have hallucinogenic properties and chemical analyses suggest certain substances present that are shared with the hallucinogenic mushroom Psilocybe cubensis. Due to our inability to use pure reference compounds and scarce amount of sample for compound identification, however, our analyses were not able to determine conclusively the presence of hallucinogenic substances.

Keywords. Basidiolichens, Huaorani, nêndapé, taxonomy.

Species containing psychoactive substances are widespread among the higher Fungi (Courtecuisse & Deveaux 2004; Gartz 1992; Guzmán 1983, 2005; Guzmán et al. 2000; Stamets 1996). The best-known examples are Claviceps spp. (Clavicipitaceae) in the Ascomycota and the genera Amanita (Pluteaceae), Psilocybe (Strophariaceae), Conocybe (Bolbitiaceae), Copelandia and Panaeolus (Coprinaceae), and Inocybe (Cortinariaceae) in the Basidiomycota, all of which contain hallucinogenic substances such as ibotenic acid, muscimol, psilocin and psilocybin (Becker et al. 1999; Centre for the Assessment and Monitoring of New Drugs 2000; Hasler et al. 2004; Hoffmann et al. 1958, 1959; Isacson et al. 1984; Leary et al. 1963, 1965; Passie et al. 2002). The use of these fungi in ancient and modern cultures over the past 3,000 years is well-documented, especially in the Mayan culture of Mexico and Guatemala and in northern Africa and the Mediterranean (Akers et al. 2011; Dugan 2008a,b; Gerault & Picart 1996; Gossop 1993; Heim 1957; Hillebrand et al. 2006; Matsushima et al. 2009; Samorini 1992; Studerus et al. 2011; Supprian et al. 2001).

Hallucinogenic lichens have rarely been reported in the literature, but there are some published accounts suggesting that certain lichens may exhibit hallucinogenic properties. For example, Parmelia saxatilis (L.) Ach., Parmotrema andinum (Muüll. Arg.) Hale, Ramalina siliquosa (Huds.) A.L. Sm. and Xanthoparmelia conspersa (Ehrh. ex Ach.) Hale have been reported to be smoked, either alone or in combination with tobacco, by members of native cultures of North America, the Shetland Islands, and Mauritania; the effects are sometimes described as stimulatory or hallucinogenic (Curtin 1949; Low 1879; Vestal & Schultes 1939; Lange 1957; Richardson 1988; Lipp 1995; Hawksworth 2003).

This paper concerns a species of Dictyonema s.lat. discovered by Davis & Yost (1983), who reported that it
was used in shamanistic rituals by the Waorani (Huaorani) people of Amazonian Ecuador and that it may have hallucinogenic properties. The Huaorani are native Amerindians living in the Amazonian forest of the provinces of Napo, Orealla and Pastaza in eastern Ecuador, with a total population size of 1,700 to 2,500 people (Beckerman et al. 2009; Lu 1999, 2001, 2005, 2006; Lu et al. 2010; Rival 2002; Yost 1981). Their Huaorani (‘huao tetedo’) language is unrelated to any other known language (Campbell 1997; Gordon 2005; Pike & Saint 1988; Rival 2002). Originally hunters and gatherers, some communities have adopted a more western lifestyle engaging in tourism, whereas others continue to live in isolation, having significant impact on wildlife populations mainly through hunting (Lu et al. 2010; Redford & Robinson 1991). The Waorani originally occupied an area of 20,000 km² before first contact by outsiders in 1958; however, their remaining lands, assigned to them in the 1990s, cover about 6,800 km² adjacent to Yasuní National Park (Rival 2002). These lands have recently been threatened particularly by oil exploration (Doughty et al. 2010; Kane 1993, 1994; Keefe 2012).

According to Davis & Yost (1983), the unidentified lichen, called ‘nênendape’, a name applied generally to fungi and lichens by the Huaorani, was used together with various bryophytes in a shamanistic ritual; a “bad shaman ate it to send a curse to cause other Waorani to die” (Davis & Yost 1983: 293). It was prepared as an infusion and apparently caused headaches and confusion when drunk. According to Davis & Yost (1983), the lichen was also used to cause sterility, given as drink to children. A similar account is given on the specimen label (see below). A Google search performed on October 9, 2014 on the terms ‘nênendape’ and ‘nenendape’ returned a total of 1 and 3,630 hits, respectively, in which the potential use of this lichen as hallucinogenic is discussed.

Apparently, the lichen is extremely rare and to date, only the single, aforementioned collection is known. However, Ott (1996) mentions the possibility that the same lichen might have been used for similar purposes by the Yurimagu Indians of Amazonian Peru, but also lists *Psilocybe yungensis* as a mushroom supposedly occurring in the same ecoregion and habitats.

The late Dr. Mason E. Hale Jr. (Smithsonian Institution) identified the lichen as a possibly undescribed *Dictyonema* species based on Davis & Yost 1051 at Harvard University (FH). *Dictyonema* s.lat. is a collective genus of lichenized Basidiomycota, for a long time believed to be small in species numbers and representing a member of Thelephoraceae (Parmasto 1978). It has been variously classified in the Aphyllorales and Polyporales, but is now known to belong in Hygrophoraceae in Agaricales (Lawrey et al. 2009). Molecular phylogenetic studies suggest now that the group consists of five distinct genera containing hundreds of species (Chaves et al. 2004; Dal-Forno et al. 2013; Lawrey et al. 2009; Lücking et al. 2014; Yáñez et al. 2012). The five genera are distinguished by morphological and anatomical features: *Cyphellostereum* and *Dictyonema* s.str. are filamentous, with simple hyphal sheaths in the first and jigsaw-puzzle-shaped enclosing cells in the second; *Acantholichen* is microsquamulose with distinct spiny hyphae on the surface (known as acanthohyphidia); and *Cora* and *Corella* are macrosquamulose to foliose with rounded lobes.

Re-study of the collection cited by David & Yost (1983) and housed at FH revealed an anatomy that coincides to some extent with both *Dictyonema* and *Cyphellostereum*, so we attempted to obtain molecular data to clarify its position in the clade. Our investigations established that it is a species of *Dictyonema*, which agrees with the rain forest ecology of the type collection; species of *Cyphellostereum* are more common in tropical montane or extratropical regions. The material could not be identified with any known species and is here described as new.

**Materials and Methods**

**Taxon sampling.** To determine the phylogenetic position of the species, we added one new sequence from the type specimen to a three-locus (ITS, nuLSU, RPB2) dataset used in a previously published phylogeny of the group (Dal-Forno et al. 2013). The entire dataset included 31 species and 81 sequences (*Table 1*).

**Morphological analysis.** Morphological characters were analyzed under the stereoscope (Olympus BX40, Nashua, NH and Stereostar Zoom Stereoscopic, Reichtert, Austria) following standard procedures for the genus (Lücking et al. 2013). Anatomical characters of the thallus were investigated by light microscopy (Olympus SZX9, Nashua, NH and Nikon Optiphot, Japan) on hand-cut sections mounted in water. Microscopic measurements were made at 1000× magnification in water.

**Molecular data.** Genomic DNA was extracted from a 0.5 cm² piece of lichenized thallus of the type specimen using the Bio 101 Fast DNA Spin Kit for tissue (Qiogene, Illkirch, France) according to the manufacturer’s protocol. About 10 ng of extracted DNA were subjected to a standard PCR in a 20 mL reaction volume using Taq Gold polymerase (Applied Biosystems, Foster City, CA), also according to manufacturer’s protocols, with the objective of amplifying the nuclear ribosomal internal transcribed spacer (ITS). The products were purified with magnetic beads (Agencourt Bioscience, Beverly, MA) and the purified PCR products were used...
in standard sequencing reactions with BigDye Terminator Ready Reaction Mix v3.1 (Applied Biosystems). The sequencing reactions were then purified using Sephadex G-50 (Sigma-Aldrich, St. Louis, MO), dried in a speedvac, denatured in HiDi Formamide (Applied Biosystems) and run on an ABI3130-xl capillary sequencer (Applied Biosystems). The data collected were analyzed using ABI software, and the sequences were then assembled together with the software Sequencher version 5.0 (Gene Codes, Ann Arbor, MI) for manual corrections in base calling and to make contiguous alignments of overlapping fragments. The primers used for sequencing were ITS1F, ITS2, ITS3, ITS4 and ITS5 (Gardes & Bruns 1993; White et al., 1990), but high quality sequences were obtained only from ITS1 and partial 5.8S, amounting to 348 bp. Amplification of other markers (nuLSU and RPB2) was attempted, but we failed to obtain products.

**Phylogenetic analysis.** The newly generated ITS sequence was assembled with sequences from GenBank belonging to 30 previously studied taxa (Table 1) using BIOEDIT 7.09 (Hall 1999) and automatically aligned with MAFFT using the --auto option (Katoh & Toh 2005). The alignment was subjected to analysis of ambiguously aligned regions using the GUIDANCE webserver (Penn et al. 2010a,b) and regions aligned with low confidence (below 0.90) were removed. This resulted in an alignment length of 2948 bases. The alignment was subjected to maximum likelihood (ML) search using RAxML 7.2.6 (Stamatakis et al. 2005; Stamatakis 2006), with non-parametric bootstrapping using 500 replicates under the universal GTR+GAMMA model.

**Chemical analyses.** A 13.6 mg sample from the 1981 sample of *Dictyonema huaorani* was ground and extracted with 1 ml of methanol-dichloromethane (50:50). Filtration and evaporation yielded ~1.0 mg of extract, which was examined using a variety of liquid chromatography/mass spectrometry (LC/MS) techniques in several different laboratories, including a preliminary analysis performed on an Agilent 1200 Series HPLC (PFP column, 100 × 4.6 mm, 2.6 µ; 0.5 mL/min; 2% to 98% MeCN/H₂O with 0.1% formic acid in 40 min) / 6130 Series mass spectrometer (low resolution).

A subsequent analysis used two different protocols (Waters, Synapt G2-S parameters: positive ion electrospray,
MS spectrum from \( m/z = 50 \) to 600, capillary voltage: positive Ion = 2.5 kV, Cone Voltage: positive Ion 30 V, source temperature = 120°C, desolvation temperature = 400°C, desolvation gas flow = 8000 L/hr, cone gas flow = 100 L/hr and Waters, Acquity parameters: column: Waters UPLC BEH C18, 2.1 × 100 mm, 1.7 μm at 45°C, injection: 5 μL, flow rate: 0.4 mL/min, gradient LC method: A = 0.1% formic acid in water; B = 0.1% formic acid in acetonitrile: A with 5% B for 0.5 min, to 95% B at 9.5 min, then 95%B for 2 min).

An alternative analysis using two different LC/MS protocols on different equipment was carried out, using Agilent 6530 QTOF LC/MS with dual spray ESI source using positive ion mode, 275°C gas temperature, 12 l/min of drying gas (nitrogen) and 35 psi of nebulizer gas (nitrogen), 3500 V capillary voltage, 50–1200 mass range, acquisition rate of 4 spectra/sec, a reference mass of \( m/z \) 922.0098, collision energy of 10, 20, and 40 V combined, mass range of \( m/z \) 25–1000, acquisition rate of 4 spectra/sec, a reference mass of \( m/z \) and an infusion of 1% acetic acid at 2 μl/min.

**Results**

**Phylogenetic analyses.** Our phylogenetic tree inferred by maximum likelihood (Fig. 1) shows *Dictyonema huaorani* placed within the paraphyletic genus *Dictyonema* s.str. Although the backbone support for this grade is low, the new species is not closely related to other lineages with semi-circular, sheet-like thalli, such as the neotropical *D. sericeum* s.str. complex and the paleotropical *D. irpicinum*. However, it is deeply nested within *Dictyonema* s.str. and bears no close relationship with *Cyphellostereum*, in spite of its reduced hyphal sheath around the filaments.

**Chemical analyses.** The preliminary analysis performed on an Agilent 1200 Series HPLC showed a peak with the same molecular weight (160 Da), UV spectrum and retention time (12.6 min) as tryptamine. Trace amounts of tryptamine can be found in mammal brains, where it is considered to play a role as a neurotransmitter and serotonin-releasing agent (Jones 1982). The subsequent, refined analysis on the Waters showed tryptamine (\( t_R 2.69 \)), 5-MeO-DMT (\( t_R 6.14 \)) and psilocybin (\( t_R 5.79 \)) with identifications based on database values. Psilocybin is one of the most widespread psychoactive fungal substances, found in more than 200 species of mushrooms. However, these are tentative identifications as no authentic standards for these compounds were available. The alternative analysis on the Agilent 6530 QTOF LC/MS with dual spray ESI source showed the likely presence of 5-methoxy-N-methyl-tryptamine, 5-methoxydimethyl-tryptamine, and 5-methoxy tryptamine, but again these identifications were based on database values, not authentic samples. All together, the results are suggestive of the presence of tryptamine and psilocybin in *Dictyonema huaorani*, but comparison of larger amounts of fresh material of this species with authentic standards are necessary to confirm these tentative findings.

**Taxonomy**

*Dictyonema huaorani* Dal-Forno, Schmull, Lücking & Lawrey, sp. nov.

**GenBank ITS barcoding type sequence:** KM208881

**Differenting from the paleotropical *Dictyonema ligulatum* (Krem.) Zahlbr. in the absence of clamps and the simple hyphal sheath consisting of irregular, branched hyphae, but not forming puzzle-shaped cells.**

**Type: Ecuador. Province Napo.** Confluence of Quivado and Tiwaeno Rivers; 28 April 1981, E. Wade Davis & Jim Yost 1051, Tomo & Kento (holotype: FH1). The label states: “Fungus growing on rotting wood. White below aquamarine above. Used by bad shaman to send a curse to cause another shaman to die. Last reported in active use four generations ago. Reported to cause severe headache in Shaman. May have hallucinogenic properties. Also said to have been used to cause barrenness. Put into a childs drink to cause sterility. May be post hoc explanation of why some women were sterile. Used with various Bryophyta (Davis 1050).”

**Description.** Thallus lignicolous on branch, filamentous, attached to the bark in the center and forming shelves on both sides, large, about 19 × 9 cm in size, green to aquamarine fibrils resting on top of a thick, white hypothallus, extending to form a distinct white yellowish prothallus. Some parts of the thallus have reddish areas which may be a result of herbarium discoloration since red is not mentioned in the original description. Thallus in section (0.3–)10.5–0.69 mm thick, composed of a thin upper photobiont layer, (80–)100–240(–311) μm thick, and a lower medulla (forming the hypothallus), (170–)300–500 μm thick; photobiont layer composed of numerous cyanobacterial filaments, wrapped in a hyphal sheath, loosely attached to the hypothallus; medulla composed of a loose network of highly interwoven hyphae. Cyanobacterial filaments more or less horizontally orientated,
composed of (7–)9(–11) μm wide and (3–)5.5(–10) μm high, blue-green cells, unbranched; heterocytes sparse to frequent, hyaline, (2–)5(–7) μm wide and (4.5–)6(–7) μm high. Hyphal sheath (2–)3.5(–5) μm wide (n = 100), hyaline, formed by densely arranged, irregular, frequently branched hyphae, mostly oriented longitudinally. Hypothallus and prothallus formed by (4–)7(–8) μm wide (n = 41), hyaline, more or less straight hyphae with mostly perpendicular branching pattern. Clamps not observed on the medullary hyphae.

Hymenophore developed as corticioid patches from the underside of the thallus margins, whitish yellow; hymenophore in section (77–)103(–124) μm thick, composed of a paraplectenchymatous layer connected to loose medullary hyphae; hymenium composed of numerous, palisade-like basidioles (15.5–)18(–25) × (6.5–)7(–8) μm; basidia and basidiospores not observed.

Chemistry. K–, C–, KC–. No conclusive chemical substances detected by liquid chromatography/mass spectrometry (LC/MS).

Etymology. The epithet is named after the indigenous tribe that has been reported to use the species in its shamanistic rituals. It is used as a noun in apposition.

Distribution and ecology. The new species is only known from the well-developed type collection from eastern Ecuador, in the northwest part of the Amazon. Despite the fact that we do not have further information about the type locality, this morphotype of *Dictyonema*, if occurring in lowland areas, usually grows under high luminosity such as restinga and introduced guava forest.
We therefore hypothesize that this species can be found in the forest canopy of the dense Amazon forest only, similar to other species of *Dictyonema* with the same morphotype that were collected in a dense humid secondary rainforest in Cantón San Lorenzo, Ecuador. This could explain why this species has been collected only once to date.

**DISCUSSION**

Davis & Yost’s (1983) study, together with other works (Hawksworth 2003; Ott 1996), suggests that hallucinogenic lichens might have been used by indigenous people for many centuries. A number of these species apparently exist and others probably remain to be discovered and investigated. The new species *Dictyonema huaorani* is a likely candidate species for these investigations and we plan to obtain fresh material for more detailed chemical analysis. Analyses of the >30-year-old specimen indicated that hallucinogenic compounds may be produced by the species, but the results remain tentative, since no authentic samples of the target substances were available for comparison. The available material, a single, though well-developed specimen, is also too limited to permit more thorough chemical studies.

Morphologically, *Dictyonema huaorani* is a typical representative of *Dictyonema s.str.* This genus is currently recognized although it appears phylogenetically paraphyletic (Dal-Forno et al. 2013; Lawrey et al. 2009). However, Lawrey et al. (2009), using the Shimodaira-Hasegawa (SH) test to compare constrained and unconstrained maximum likelihood trees, showed that monophyly of *Dictyonema s.str.* cannot be rejected. Anatomically, the new species also agrees with *Cyphellostereum* in the simple hyphal sheath around the cyanobacterial filaments and the comparatively narrow filament cells (Chaves et al. 2004; Dal-Forno et al. 2013; Yáñez et al. 2012), but the molecular phylogenetic data confirm its placement in *Dictyonema s.str.* It most closely resembles *Dictyonema ligulatum* from the Paleotropics (Lücking et al. 2013), but the latter differs in having clamps and broader filaments with a puzzle-shaped hyphal sheath. Both species differ from other species of *Dictyonema* with shelf-like growth of the very

![Figure 2. Habitus of Dictyonema huaorani (holotype). A. Upper side of lichen thallus. B. Detail of upper thallus lobe. C. Lower side of lichen thallus. D. Detail of lower thallus, showing the hymenophore.](image)
compact thalli with an almost smooth surface (Lücking et al. 2013). The molecular data place the new species in *Dictyonema* s.str., but outside *D. sericeum* in which it was originally suggested to belong.

Recent studies by our working group have made clear that clades in *Dictyonema* s.lat., including *Cora* and *Corella* (Lücking et al. 2014), *Acantholichen* (Dal-Forno et al. in prep.) and *Dictyonema* s.str. (Dal-Forno et al. in prep.) are far more species-rich than anyone could have reasonably predicted in the past. For example, over 120 morphologically and genetically distinct new species of *Cora* were recently discovered, with a predicted number well over 400 species (Lücking et al. 2014). Lichens in this clade were once thought to be members of a single species, *Dictyonema glabratum* (Sprengel) D. Hawksw. (also known as *Cora pavonia* (Sw.) Fr.). They are mostly large, familiar, commonly observed and frequently collected, so the discovery of so much undescribed diversity was entirely unexpected, certainly one of the most spectacular cases of undescribed diversity in a common macroorganism. This study makes clear the need to establish biologically meaningful species recognition guidelines for this and other groups in *Dictyonema* s.lat., and also for increased and targeted collecting in biodiversity hotspots predicted to support and maintain undescribed species. Up to now, much of this newly-discovered diversity is neotropical, but for *Dictyonema* s.str., undescribed species undoubtedly exist also in the Paleotropics, where collecting efforts have so far been more diffuse and far less intensive. Given the interesting and unusual ecology and chemistry of these species, investigators will, we hope, make collecting them a higher priority.

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