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**Article**

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# Two new neotropical species of the ectomycorrhizal gasteroid genus *Calostoma* (Sclerodermatineae, Boletales) used as a food source by the *Ayuuk jä'äy* ethnic group from Southern Mexico

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## Abstract

During a study of fungal species with biocultural importance in the Sierra Mixe in the state of Oaxaca, Mexico, specimens belonging to the genus *Calostoma* were collected in 2015. Morphological characteristics, electron micrographs and phylogenetic analyses of the collected specimens were used to identify the fungi at the species level. Herein we describe *Calostoma naaxtutus* and *Calostoma tooteic* as new species and compared them morphologically and phylogenetically with their most similar species, *C. cinnabarinum*. This constitutes one of the few records of any mushroom species that the Mexican *Ayuuk jä'äy* ethnic group uses.

**Key words:** Boletales, edible fungi, ethnomycology, ethnotaxa, Mixe people, mycocultural heritage, Oaxaca

## Introduction

*Calostoma* Desvaux (1809), a genus with American and Easter Asiatic distribution (Wilson *et al.* 2012) includes 26 species (Index Fungorum 2017), of which three (*C. junghuhnia*, *C.* cf. *fuscum* and *C. cinnabarinum*) are used as edible and medicinal mushrooms by different ethnic groups in Thailand, Malaysia and Mexico (Abdullah & Rusea 2009, Alatorre 1996, Bandoni *et al.* 1998, Bautista-Nava & Moreno-Fuentes 2009). The ectomycorrhizal life strategy has been described only for three species of the genus in association with Fagaceae, Myrtaceae, and Nothofagaceae (Wilson *et al.* 2007, 2012). The genus *Calostoma* belongs to the family Calostomataceae, suborder Sclerodermatinae (Binder & Bresinsky 2002), and order Boletales (Gilbert 1931), according to their ribosomal, mitochondrial and nuclear DNA sequences (Hughey *et al.* 2000, Hibbett *et al.* 2000). The family Calostomataceae is characterized by an apical ostiole with raised vertices of variable edges, similar to a star, which confers the name to the genus, derived from the Greek kallos=beautiful, and stoma=mouth, literally meaning "beautiful mouth" (Desvaux 1809, Ulloa & Herrrera 1994). The type species of the genus *C. cinnabarinum* Desvaux (1809) is ectomycorrhizal and forms a pigment called calostomal (Gruber & Steglich 2007). This species has been reported in several countries in the Americas (Wilson *et al.* 2007, Baseia *et al.* 2007, Calonge *et al.*2005, Arzu *et al.* 2012) and in China (Wilson *et al.* 2012). The specific epithet *cinnabarinum* comes from the Ancient Greek word "kinnábari" (κιννάβαρι), which refers to its "cinnabar-red" color of the spherical

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head of the species. Mexico is a centre of biocultural diversity with 65 ethnic groups, and one of these ethnic groups is called the *Ayuuk jä'äy* (or Mixe culture), a pre-Hispanic community of around 130,000 indigenous people (INEGI 2015) that originated in approximately 2,000 years ago, being a putative successor of the Olmec ancient Mesoamerican culture (Barros 2006). This group lives in a region located in the westernmost part of the northern highlands of the state of Oaxaca, in a region called Sierra Mixe (literally Mixe=mountain range) and so far, no formal reports of the mushrooms that they use have been recorded. The objective of this work was to taxonomically characterize the species of the genus *Calostoma* existing in the Sierra Mixe using morphological characteristics, electron microscopy and phylogenetic analyses. In addition, we determined if these species are eaten by the *Ayuuk jä'äy* community.

## Materials & methods

### *Field work*

From June to September 2015, 18 field visits were made to the municipality of Santa María Tlahuitoltepec, located in the Sierra Mixe of the state of Oaxaca, Mexico. Open-ended interviews were conducted with local *Ayuuk jä'äy* speakers. These interviews were related to consumption, location and biocultural importance of the specimens whose description coincided with species of the genus *Calostoma*. A factor that facilitated the collection of specimens and information on the biocultural importance of these species was that the third co-author of the present work is a native speaker and reader-writer of the *Ayuuk* language and is native to the area where this study was carried out. Subsequently, random walks were made in forested areas reported to look for the taxa recorded in the interviews. Basidiomes of species of the genus *Calostoma* were photographed and collected at different sites, which were georeferenced.

### *Morphological description*

The morphological description of the macroscopic characteristics such as the colour, size and texture of the peridium, stipe, and gleba were made *in situ*. Specimens to be used as voucher collections for herbarium were dehydrated in a mushroom drier at 40°C (Hu *et al.* 2022). We took small samples of the basidiomata in the field, which is located in a remote mountainous area in Mexico, using sterile scalpels and gloves on a sterile mat, immediately after being collected. Then, these samples were placed on sterile 1.5 mL Eppendorf tubes (Thermo Fisher Scientific Inc., Waltham, Massachusetts. USA) containing silica gel. This process allowed to slowly extract the humidity of the samples, while they were transported to the laboratory conditions, where DNA extraction was successfully conducted. Next, once in the lab, a description of the microscopic characteristics was made, including the shape, size and ornamentation of the spores and characteristics of the peridium. For practical purposes, the size and shape of the basidiospores were analysed in the format of the biometric variables recorded by Tullos & Yang (2017). Herbarium specimens were deposited in the National Autonomous University of Mexico (MEXU). The identification of the dominant oak arboreal species was performed by Enrique Guizar Nolazco of the Universidad Autónoma Chapingo, Mexico, who is a specialist in the genus *Quercus* L. The spores of the mature studied specimens were mounted on Melzer's reagent, lactophenol blue, and 5% KOH, and analysed under light microscope for micromorphological characterization. Microscopic characters were measured with an optical microscope (Carl Zeiss GmbH 37081, Germany). Differences in the spore ornamentation of mature basidiomata, were observed. Then, in order to have more accurate information related with the spore ornamentation they were subjected to scanning electron microscopy in Colegio de Postgraduados, Mexico, as described below. Spores of mature specimens were mounted on bronze specimen holders with the aid of copper double-sided adhesive tape. These samples were sputter-coated with gold and palladium using a fine coat ion sputter (JFC-100, JEOL, Tokyo, Japan). Metallized spores were observed in the JSM-6390 (JEOL) scanning electron microscope at the Colegio de Postgraduados, Mexico.

### *DNA extraction and phylogenetic analyses*

DNA extraction was carried out in the Biotechnology Laboratory of the Center for Research and Advanced Studies of the National Polytechnical Institute (Instituto Politécnico Nacional, CINVESTAV) Zacatenco from a 1 cm portion of peridium and gleba of four mature specimens selected for their macro and micromorphological characteristics from the different sampling sites. The tissue was disintegrated by freezing in liquid nitrogen and mechanical disruption using TissueLyser LT in three-minute cycles. The extraction was performed using a PowerSoil ® DNA Isolation Kit, and 1% agarose gels were used to verify the quality of the DNA. The internal transcribed spacer (ITS) fragments were obtained by PCR using the following reaction mixture with the TaKaRa Ex Taq ™ reagents: 2 µl of buffer, 1.6 µl of dNTPs, 0.06

µl of Ex Taq, 0.4 µl of the ITS4 and 0.4 µl of the ITS5 oligos at a concentration of 10 µM, 2 µl of DNA and 13.54 µl of $H_2O$. Once the amplification was confirmed via agarose gel electrophoresis, the DNA concentration was measured using a NanoDrop™ 2000/2000c and then samples were capillary-sequenced by a Biotechnology and Prototype Unit (UBIPRO) at the Department of Higher Education (Facultad de Estudios Superiores, FES) Iztacala, UNAM.

### *Phylogenetic analyses*

To explore the phylogenetic relationships of the new species, an alignment was made based on the taxonomic sampling employed by Xu *et al.* (2022). The ITS region was aligned using the online version of MAFFT v7 (Katoh *et al.* 2002; 2017; Katoh & Standley 2013). Alignments were reviewed in PhyDE V. 10.0 (Müller *et al.* 2005), followed by minor manual adjustments to ensure character homology between taxa. The matrix was composed of 56 taxa (690 characters). *Gyroporus castaneus* (access number EU819468) was used as the outgroup. Phylogenetic inferences were estimated with maximum likelihood in RAxML v. 8.2.10 (Stamatakis 2014) with a GTR + G model of nucleotide substitution. To assess branch support, 10000 nonparametric rapid bootstrap pseudoreplicates were run with the GTRCAT model. For Bayesian posterior probability, the best evolutionary model for alignment was sought using Partition Finder v.2 (Lanfear *et al.* 2014; 2017; Frandsen *et al.* 2015). Phylogenetic analyses were performed using MrBayes v. 3.2.6 x64 (Huelsenbeck & Ronquist, 2001). The information block for the matrix included two simultaneous runs, four Montecarlo chains, temperature set to 0.2 and sampling 10 million generations (standard deviation ≤0.1) with trees sampled every 1000 generations. The first 25% of samples were discarded as burn-in, and stationarity was checked in Tracer v. 1.6 (Rambaut *et al.* 2014). Trees were visualized and optimized in FigTree v. 1.4.4 (Rambaut 2014), and they edited in Adobe Illustrator vCS4 (Adobe Systems, Inc., San Jose, CA).

## Results

### *Phylogenetic analyses results*

Sequences produced in this study of *C. naaxtututs* and *C. tooteic* were deposited in GenBank (Table 1). The two simultaneous Bayesian runs continued until the convergence parameters were met, and the standard deviation fell below 0.0001 after 10 million generations. No significant changes in tree topology trace or cumulative Split frequencies of selected nodes were observed after about 0.3 million generations, so the first 2,500,000 sampled trees (25%) were discarded as burn-in. The analysis of ITS produced a phylogenetic tree where *Calostoma* is shown as a monophyletic clade (1 Bayesian Posterior Probability (PP) and 100% bootstrap proportion (BP) for Maximum Likelihood). Both the Bayesian analyses and Maximum Likelihood (Fig. 1) recovered *C. naaxtututs* and *C. tooteic* supporting the existence of two new taxa distinctive from related species of *Calostoma.*

**TABLE 1.** Names, voucher numbers, localities, references and corresponding GenBank accession numbers of the sequences used in the phylogenetic analyses. Newly generated sequences are in bold.

| Taxon name | Voucher | Locality | GenBank Accession ITS | References |
|---|---|---|---|---|
| *Calostoma areolatum* | HMJAU45266 | China | MG841353 | Liu *et al.* (2018) |
| *Calostoma areolatum* | HMJAU45266 | China | MG871352 | Liu *et al.* (2018) |
| *Calostoma areolatum* | HMJAU45266 | China | MG871351 | Liu *et al.* (2018) |
| *Calostoma areolatum* | FHMU2089 | China | OK157456 | Xu *et al.* (2022) |
| *Calostoma areolatum* | FHMU2093 | China | OK157457 | Xu *et al.* (2022) |
| *Calostoma areolatum* | FHMU2096 | China | OK157458 | Xu *et al.* (2022) |
| *Calostoma berkeleyi* | AWW268 | Malaysia | EU718090 | Wilson *et al.* (2012) |
| *Calostoma berkeleyi* | JFK77 | Malaysia | FJ710189 | Wilson *et al.* (2011) |
| *Calostoma cinnabarinum* | AWW136 | USA | AY854064 | Wilson *et al.* (2011) |
| *Calostoma fuscum* | PDD70777 | ----- | FJ710190 | Liu *et al.* (2018) |
| *Calostoma fuscum* | OKM23918 | Australia | EU718091 | Wilson *et al.* (2011) |
| *Calostoma insigne* | Arora 98-31 | Thailand | EU718092 | Wilson *et al.* (2011) |
| *Calostoma japonicum* | OKM22412 | Japan | FJ710191 | Wilson *et al.* (2011) |

......*continued on the next page*

**TABLE 1.** (Continued)

| Taxon name | Voucher | Locality | GenBank Accession ITS | References |
|---|---|---|---|---|
| *Calostoma japonicum* | TKG-SC-40701 | Japan | EU718093 | Wilson *et al.* (2011) |
| *Calostoma japonicum* | 153-196 | ----- | AB509607 | Unpublished |
| *Calostoma lutescens* | 1329 | ----- | FJ710192 | Wilson *et al.* (2011) |
| *Calostoma lutescens* | Utleg750 | Costa Rica | JX184406 | Trierveiler-Pereira *et al.* (2013) |
| *Calostoma lutescens* | AM1208 | USA | JX184404 | Trierveiler-Pereira *et al.* (2013) |
| *Calostoma lutescens* | NY223 | USA | JX184405 | Trierveiler-Pereira *et al.* (2013) |
| *Calostoma miniata* | HKAS41452 | China | FJ710194 | Liu *et al.* (2018) |
| *Calostoma miniata* | HKAS41023 | China | FJ710193 | Liu *et al.* (2018) |
| ***Calostoma naaxtututs*** | **MEXU 29001 Type** | **Mexico** | **MF521438** | **This study** |
| ***Calostoma naaxtututs*** | **MEXU 29008** | **Mexico** | **MF521440** | **This study** |
| *Calostoma oriruber* | HKAS32119 | China | FJ710195 | Wilson *et al.* (2011) |
| *Calostoma oriruber* | HMAS46524 | China | FJ710196 | Liu *et al.* (2018) |
| *Calostoma ravenelii* | 510 | USA | EU718094 | Wilson *et al.* (2012) |
| *Calostoma ravenelii* | 462 | USA | FJ710197 | Wilson *et al.* (2011) |
| *Calostoma ravenelii* | AJR04 | USA | MF755286 | Liu *et al.* (2018) |
| *Calostoma retisporum* | Mka9 | Malaysia | FJ807565 | Wilson *et al.* (2012) |
| *Calostoma retisporum* | Mka4 | Malaysia | FJ807564 | Wilson *et al.* (2012) |
| *Calostoma retisporum* | AWW354 | Malaysia | FJ807563 | Liu *et al.* (2018) |
| *Calostoma rodwayi* | GMM7572 | New Zealand | EU718095 | Wilson *et al.* (2011) |
| *Calostoma rodwayi* | PDD69216 | ----- | FJ710198 | Wilson *et al.* (2011) |
| *Calostoma sarasinii* | AWW244 | ----- | FJ710199 | Wilson *et al.* (2011) |
| *Calostoma sarasinii* | DED7660 | Malaysia | EU718096 | Wilson *et al.* (2012) |
| *Calostoma sarasinii* | FH48 | Malaysia | FJ807562 | Wilson *et al.* (2012) |
| *Calostoma sarasinii* | FH25 | Malaysia | FJ807561 | Wilson *et al.* (2012) |
| *Calostoma sarasinii* | FH21 | Malaysia | FJ807560 | Liu *et al.* (2018) |
| *Calostoma sarasinii* | FH1 | Malaysia | FJ807559 | Liu *et al.* (2018) |
| *Calostoma sinocinnabarinum* | FHMU3739 | China | OK157459 | Xu *et al.* (2022) |
| *Calostoma sinocinnabarinum* | FHMU3732 | China | OL839189 | Xu *et al.* (2022) |
| *Calostoma sinocinnabarinum* | FHMU3818 | China | OL839190 | Xu *et al.* (2022) |
| *Calostoma* sp. | HONDURAS19-F031a | Honduras | MT571527 | Unpublished |
| *Calostoma* sp. | RC-47 | ----- | MF521440 | Unpublished |
| *Calostoma* sp. | RC-46 | ----- | MF521439 | Unpublished |
| *Calostoma* sp. | RC-45 | ----- | MF521438 | Unpublished |
| *Calostoma* sp. | RC-44 | ----- | MF521437 | Unpublished |
| *Calostoma* sp. | HKAS 104346 | ----- | MG735427 | Unpublished |
| *Calostoma* sp. | HKAS38139 | China | EU718098 | Wilson *et al.* (2011) |
| *Calostoma* sp. | HKAS38133 | China | EU718097 | Wilson *et al.* (2011) |
| *Calostoma* sp. | CM13-235 | ----- | KY774026 | Unpublished |
| *Calostoma* sp. | PDD71264 | ----- | FJ710200 | Unpublished |
| *Calostoma* sp. | BB2004-222 | USA | EU543222 | Wilson *et al.* (2012) |
| ***Calostoma tooteic*** | **MEXU 29002 Type** | **Mexico** | **MF521437** | **This study** |
| ***Calostoma tooteic*** | **MEXU 29009** | **Mexico** | **MF521439** | **This study** |
| *Gyroporus castaneus* | JMP0028 | ----- | EU819468 | Liu *et al.* (2018) |

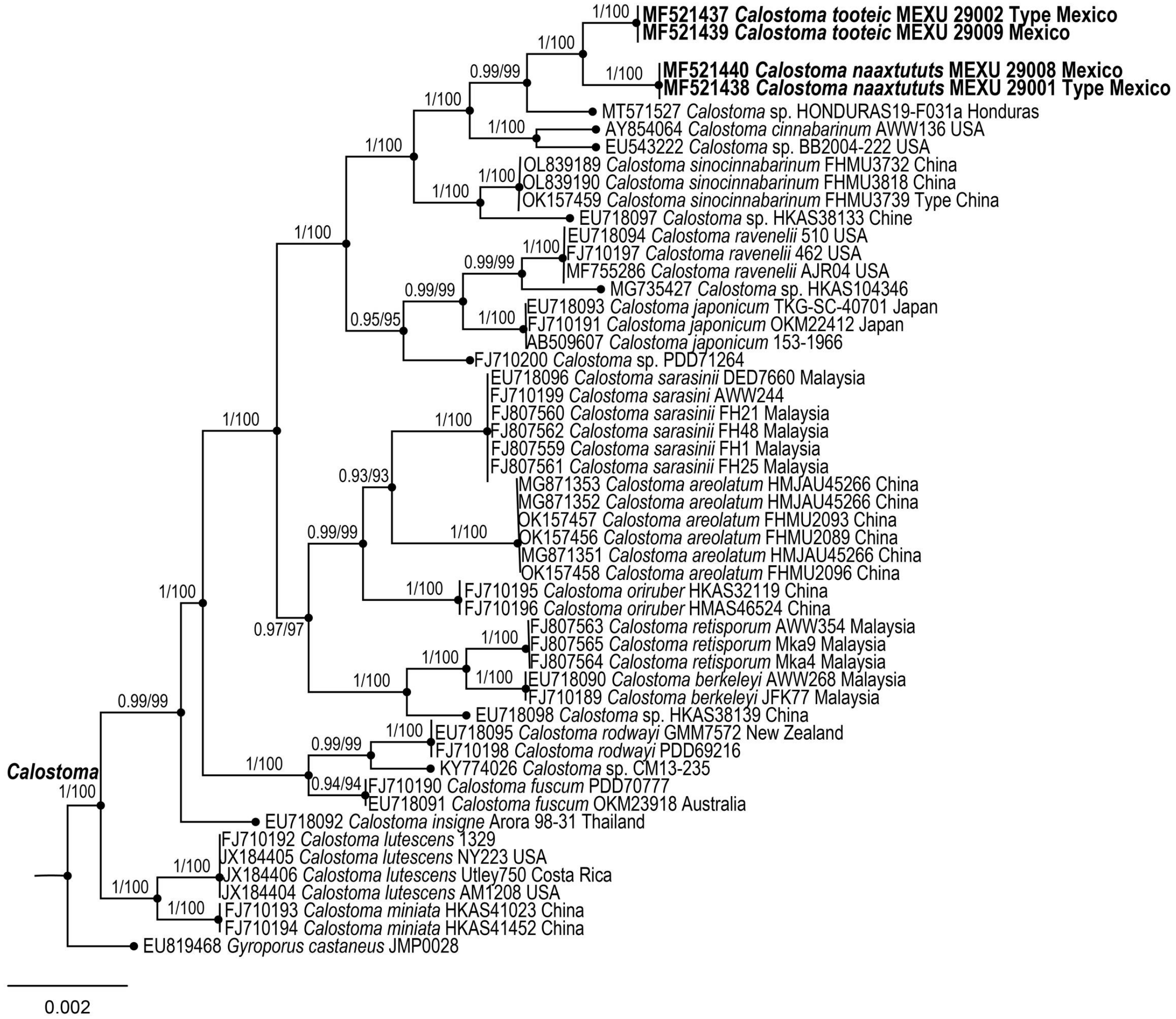

**FIGURE 1**. Phylogram of Bayesian inference (BI) tree from the ITS sequence data of 56 specimens. *Gyroporus castaneus* was used as the outgroup. The values above branches represent Bayesian posterior probabilities (PP, left) and bootstrap values (LP, right) for maximum likelihood, respectively. The scale bar represents the expected number of nucleotide substitutions per site. Sequences obtained from this study are in bold.

### *Morphology and edibility*

The collected specimens showed morphological and molecular characteristics different from the species currently described for the genus *Calostoma*. The exoperidium and height of the stipe was different among the specimens collected, forming two groups (Figure 2). The first group exhibited a thick exoperidium that broke only at the apex and could be broken in different parts but that did not cover the short stipe, which has a maximum length of 15 mm (Figure 2a, b). The other group presented a thin exoperidium that at ripening opened at the apex, and a stipe of up to 5 cm, composed of a gelatinous layer, with embedded small, orange to reddish seed-like rounded small structures (Figures 2d–e). In the same way, the size and ornamentation of the spores were different from the species described for the genus; and between the two collection groups (Figure 2c–f). The molecular comparison in GenBank revealed less than 95% similarity with the sequence of the most similar species *Calostoma cinnabarinum* (Table 1); likewise, the topology of the phylogenetic analyses of the clades was different from *C. cinnabarinum* and the collected specimens, which were grouped into the categories of with and without long stipes. These data show that in the study region, there are at least two different species that were distinct from the species already described in the genus *Calostoma* (Figure 2).

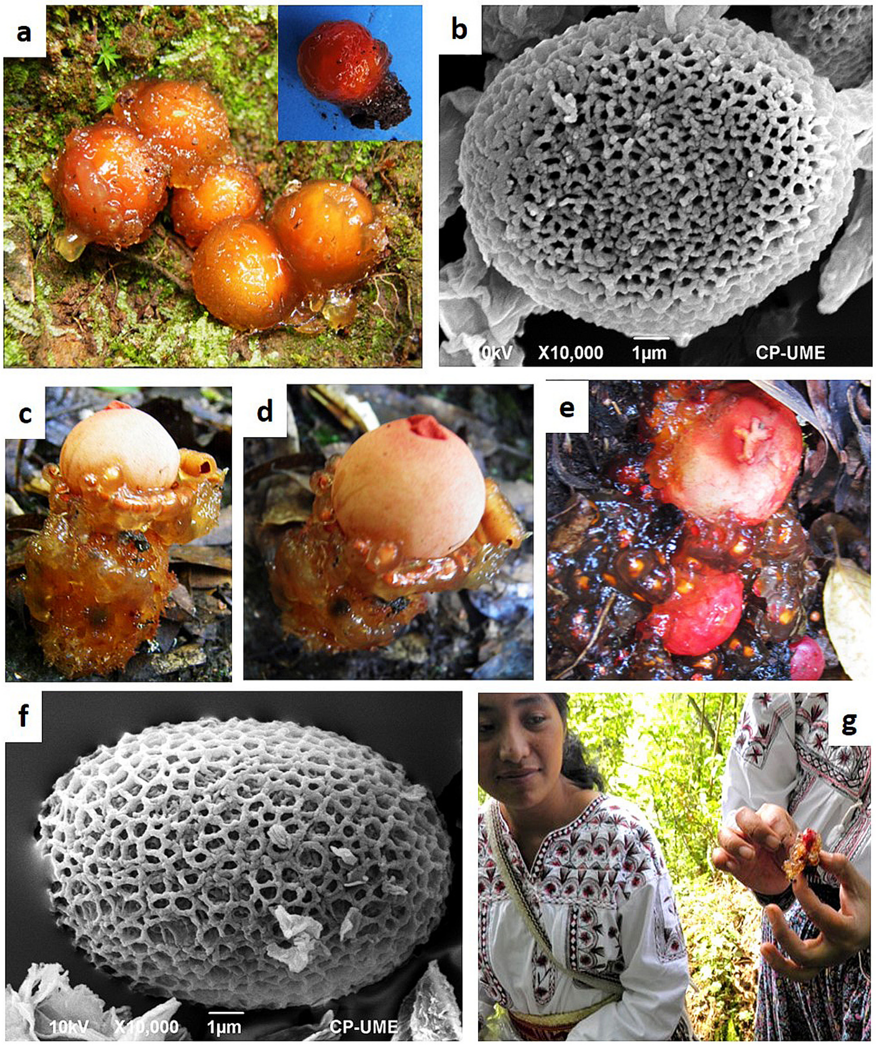

**FIGURE 2.** *Calostoma naaxtututs* (a–b) and C. *tooteic* (c–f). a. Gasterocarp appearance (Holotype), showing color variation (in the top right corner). b) Scanning electronic microphotography showing the ornamentation pattern of spores; c–d) Gasterocarp appearance (Holotype), showing colour variation in (e). f. Scanning electronic microphotography showing the ornamentation pattern of spores; (g) Gathering of *Calostoma* ethnotaxa by *Ayuuk jä'äy* women.

## Taxonomy

***Calostoma naaxtututs*** **Deloya-Olvera, Virgen-Vasquez, Xoconostle-Cázares & J. Pérez-Moreno, *sp. nov.***
*Index fungorum number: IF556486, Mycobank number: MB 830881, GenBank: MF521438 and MF521440*

***Etymology:*****—**Refers to the name used by *Ayuuk jä'äy* people to designate this edible species *naax tututs* (*naax*=earth, *tututs*=egg) literally meaning "egg of the earth" due to egg-like flavour of the fungus.

Gasterocarp in the form of a globose spore-sac with short stipe, formed by longitudinal anastomosed strands, reddish-brown in colour, cartilaginous when fresh and hard when dry, from 5 to 15 mm in height, × 24 mm in diameter. Spore-sac orange to red, 23 to 30 mm in diameter, with a gelatinous exoperidium 3 mm thick, hyaline, when ripe, it opens at the apex on the side of the ostiole. Mesoperidium reddish orange attached to the exoperidium. Endoperidium papyraceous of cream-yellowish colour. Ostiole apical star-shaped with five to six raised edges, which breaks and release the spores. Gleba creamy, whitish-yellowish. Basidiospores extensively ellipsoid (9.3) 9.9–13.8 (14.6) × (7.0) 7.3–10.8 (11.7) µm, Q= 1.08–1.71 µm; Qm = 1.27 µm, ornamented with reticulum (exospore) with a single agglomerated layer with irregular spaces which can be closed by the same reticulum (Fig. 2b). Spore-sac formed by a network of intertwined hyphae 1.5–7.8 µ in width (Figure 2).

**Holotype:**—MEXICO, Oaxaca, Santa Maria Tlahuitoltepec, on *Quercus obtusata* Bonpl. forest soil, 17°6'27.2"N, 96°3'51.5"W, 2477 m, 19 August 2015, Deloya-Olvera 51 (MEXU29001!, holotype designated here).

Paratype:—MEXICO, Oaxaca, Santa Maria Tlahuitoltepec, on *Quercus obtusata* forest soil, 17°6'27.2"N, 96°3'51.5"W, 2477 m, 19 August 2015, Deloya-Olvera 53 (MEXU29008!, paratype designated here).

**Diagnosis:** Differing from other *Calostoma* species by its thick gelatinous exoperidium, persistent, hyaline, 3 mm thick; size of basidiospores (9.3)9.9–13.8(14.6) × (7.0)7.3–10.8(11.7) µm, Q= 1.08–1.71 µm; Qm= 1.27 µm; and stipe size: 0.5–1.5 cm high × 0.3–1.0 cm in diameter.

**Notes:** *Calostoma naaxtutus* is different from its closest phylogenetic species *C. cinnabarinum* due to: i) its greater globose spore-sac; the former species has a 20–30 mm spore-sac in diameter, while in the later species the folllowing measures have been recorded: 10–20 mm; 10–15 mm; 8–10 mm and 8–12 mm by Masse (1888); Castro-Mendoza *et al.* (1983); Kim *et al.* (2007) and Baseia *et al.* (2010), respectively; ii) the ontogeny of the exoperidium, while it has been recorded that in *C. cinnabarinum,* the gelatinous exoperidium falls when mature (Coker & Couch, 1928; Castro-Mendoza *et al*. 1983; Kim *et al.* 2007), in the case of *C. naaxtutus,* the exoperidium persists even in mature specimens; iii) the longer spores reported for *C. cinnabarinum*: 15–18 µm , 14–20 µm , 12–22 µm , 10–20 µm , 12–15 µm and 13.5–16.5 µm, by Masse (1888); Coker & Couch (1928); Castro-Mendoza *et al.* (1983); Kim *et al.* (2007) and Guzmán (1973), respectively, while our specimens have spores ranging from 9.9–13.8 µm in length; iv) the shorter stipe, previously reported from 7–10cm , 2–5 cm, 3.5 cm and 1.5–2.0 cm by Desvaux (1809); Masse (1888); Castro Mendoza *et al.* (1983); and Baseia *et al.* (2007), respectively, but in our specimens the stipe ranged from 0.5–1.5 cm high; and iv) genetic differences which showed that *C. naaxtutus* is included in a monophyletic clade (1 Bayesian Posterior Probability and 100% bootstrap proportion for Maximum likelihood), closer but different to that of *C. cinnabarinum. Calostoma naaxtutus* differs from the other two species of the genus, previously recorded from Mexico, because *C. lutescens* (Schwein.) Burnap has globose spores, (6–)7.7–10.5 µm in diameter; and *C. ravenelii* (Berk.) Massee has an exoperidium which is non-gelatinous, and also has longer spores (12–)13.5–16.8(–18) µm (Guzmán, 1973).

### ***Calostoma tooteic*** **Deloya-Olvera, Virgen-Vasquez, Xoconostle-Cázares & J. Pérez-Moreno** ***sp. nov.***

*Index fungorum number: IF556487, Mycobank number: MB 830882, GenBank MF521437 and MF521439*

***Etymology:***—Refers to the name used by *Ayuuk jä'äy* people to designate the long stipe of this edible species, and the possibility to find this fungus in the rainy season; *too teic* (*too*= rain, *teic*=foot) literally meaning "the rain foot".

Gasterocarp formed by a tall stipe with a globose spore-sac at the apex; stipe is formed by longitudinal anastomosed strands and is transparent reddish brown, cartilaginous when fresh and hard when dry, from 1.5 to 5 cm in height, × 1.8 to 2.20 cm in diameter. Spore-sac, globose, orange to red, 20–28 mm in diameter, initially covered by a thin gelatinous exoperidium containing small reddish "seeds" that fall embedded in the gelatine around the spore-sac, sliding down the stipe and leaving the pore-sac completely bare; ostiole apical, with five to six raised edges; basidiospores ellipsoid (11.3–) 12.2–18.0 (–19.2) × (8.5–) 8.8–12.4 (12.8) µm, Q= 1.11–1.81 µm ; Qm=1.4 µm , ornamented with a reticulum (exospore) with internal and external layers (Figure 2f). Spore sac formed by a network of hyphae from 2.2 to 5.8 µm that degrades (Figure 2c–e).

**Holotype**:—MEXICO, Oaxaca, Santa Maria Tlahuitoltepec, on *Quercus obtusata* forest soil, 17°6'41.6"N, 96°3'33.7"W, 2327 m, 19 August 2015, Deloya-Olvera 57 (MEXU29002!, holotype designated here).

Paratype—MEXICO, Oaxaca, Santa Maria Tlahuitoltepec, on *Quercus obtusata* forest soil, 17°6'59.7"N, 96°3'23.2"W, 2477 m, 20 August 2015, Deloya-Olvera 58 (MEXU29009!, paratype designated here).

**Diagnosis:** differing from other *Calostoma* species by exoperidium gelatinous, thin and ephemeral with age; size of spores: (11.3–)12.2–18.0(–19.2) × (8.5–)8.8–12.4(–12.8) µm, Q= 1.11–1.81 µm Qm=1.4 µm and stipe 1.5–5.0 cm high × 1.5–2.0 cm in diameter.

**Notes:** *Calostoma tooteic* differs from its closest phylogenetic species *C. cinnabarinum* due to: i) its greater globose spore-sac; the former species has a 20–28 mm spore-sac in diameter, while as pointed out above in the case of *C. cinnabarinum* smaller measures have been recorded: 10–20 mm; 10–15 mm; 8–10 mm and 8–12 mm by Masse (1888); Castro-Mendoza *et al.* (1983); Kim *et al.* (2007) and Baseia *et al.* (2010), respectively; ii) the narrower spores reported for *C. cinnabarinum*: 8–10 µm, 6.3–8.5 µm, 6.5–8.5 µm, 5–8 µm, 8–10 µm and 7.5–9 µm, by Masse (1888); Coker & Couch (1928); Castro-Mendoza *et al.* (1983); Kim *et al.* (2007) and Guzmán (1973), respectively, while our specimens have spores ranging from 8.8–12.4 µm in width; and iv) as mentioned for *C. naaxtutus*, there were robust genetic differences which showed that *C. tooteic* is included in a monophyletic clade (1 Bayesian Posterior Probability and 100% bootstrap proportion for Maximum likelihood), closer but different to that of *C. cinnabarinum.* Similarly than in the case of. *C. naaxtutus, C. tooteic* differs from the other two species of the genus previously recorded from Mexico, because *C. lutescens* has globose spores, (6–)7.7–10.5 µm in diameter; and *C. ravenelii* has a non-gelatinous exoperidium (Guzmán, 1973).

## Discussion

The taxonomic history of the genus *Calostoma*, and that of its type species *C. cinnabarium* dates back to 1692. In that year the English botanist Leonard Plukenet illustrated in detail one specimen from the state of Virginia, USA which showed a globose cap with an apical ostiole with six raised vertices in its upper central part and a long-twisted stipe. Along with his draw he wrote a Latin note which said "*Fungus pulverulentus virginianus caudice coralline topario opere contorto*" denoting a dusty fungus from Virginia decorated with a coral red stipe. Since late–19th and early 20th centuries, a number of mycologists have frequently recorded this species in North America (Reed 1910). However, with the advent of molecular biology, the concept of what has been named "*Calostoma cinnabarinum*" should be carefully revised, mainly in Canada, USA and Mexico in order to have a more precise and deeper insight of the taxon. As far as Mexico is concern, Guzmán (1973) presented a description of the three species recorded in the country so far: *C. lutescens* is characterized by its globose (6–)7.5–10.5 µm in diameter; based on two specimens; *C. ravenelii* is characterized by its non-gelatinous exoperidum with (12–)13.5–16.8(–18) × (7.5–)9–10.5 µm elliptic spores; based on one specimen; and *C. cinnabarinum* is characterized by its gelatinous exoperidium with (10.5–)13.5–16.5(–21) × (6–)7.5–9(–12) µm elliptic spores; based on 13 specimens. We examined specimens collected in 1965 and 1973 in the state of Oaxaca, Mexico and deposited in the MEXU herbarium, located in Mexico City, without fresh morphological notes, reported as "*Calostoma cinnabarinum*", with variable Q values, ranging 1.53–3.16 with Qm=2.05 (MEXU4988) those collected in Huautla de Jimenez, Oaxaca and also specimens collected in Ixtlan, Oaxaca (MEXU9433) with Q=1.6–2.6 with Qm=1.99. Our work demonstrates that a precise definition of species in the genus *Calostoma* requires comprehensive polyphasic studies which must include, in addition to precise macro and micromorphology data of gasterocarps, scanning electron microscopy of the spores in order to resolve accurate spore ornamentation (as demonstrated by Castro-Mendoza *et al.* 1983; and in this work) and phylogenetic analyses in order to have a deeper insight of the species in the genus. Table 2 presents a comparative analysis of the two species described in the present work compared to *C. cinnabarinum* which is the type species of the genus and the most phylogenetically similar species with percentages of similarity from 91 to 94% with *C. tooteic* and 95 % with *C. naaxtututs*, respectively, showing diagnostic differences among the species. The species described in the present work increases the number of taxa of the genus *Calostoma* currently known in the neotropics to eight.

**TABLE 2.** Diagnostic characteristics of the new species determined in this work and *Calostoma cinnabarinum*.

| Characteristics | *C. cinnabarinum* *(*Kim *et al*. 2007) | *C. cinnabarinum* (Guzmán 1973) | *C. naaxtututs* This work | *C. tooteic* This work |
|---|---|---|---|---|
| Size of spores (µm) | 10–20 × 5–8 | (10.5–) 13.5–16.5 (–21) × (6–) 7.5–9 (12) | (9.3) 9.9–13.8 (14.6) × (7.0) 7.3–10.8 (11.7) | (11.3) 12.2–18.0 (19.19) × (8.5) 8.8–12.4 (12.8) |
| Peridium | Dark orange, 8–10 mm thick, globose to subglobose shaped | Orange-reddish | Dark orange -reddish (5A8–6A7) covered with a jelly exoperidium | Orange (5A6) covered with a gelatinous exoperidium |
| Exo-peridium | Thick, hyaline, falling at maturity, mostly with the mesoperidium | Gelatinous, hyaline with orange-reddish incrustations | Thick gelatinous, persistent, hyaline 3 mm thick | Thin gelatinous, falling at maturity |
| Size of stipe | ND | ND | 0.5–1.5 cm high × 0.3–1.0 cm in diameter | 1.5–5.0 cm high × 1.5–2.0 cm in diameter |
| Stipe | ND | Gelatinous rhizomorphic base, alveolate-lacunose, hyaline | Longitudinal strands, lacunose, reddish-brown, cartilaginous when fresh hard when dry | Strands longitudinally arranged Anastomosed, hyaline-orange, cartilaginous when fresh hard, brown-hyaline when dry |

ND= not described.

This is the third record of the edible species of the genus *Calostoma* in the world. Additionally, this is the first record of the use of *Calostoma* species as food by the *Ayuuk jä'äy* ethnic group, reported in the testimonies of 30 residents, including children less than 10 years old in a community of 199 inhabitants. The *Ayuuk jä'äy* consume it as a wild snack, eating the peridium and the gleba raw, and removing the gelatinous exoperidium and the stipe. The inhabitants refer to this food as *naaxututs* (*naax*=earth, *tututs*=egg) meaning "egg of the earth", which refers to the egg-like flavour of the fungus. Thus, based on linguistic and ethnological information we can assume that ancient traditional knowledge related to *Calostoma* species has been maintained by the members of the surviving *Ayuuk jä'äy* community for a long period of time. This ethnotaxon grows in forests dominated by *Quercus obtusata* Bonpland and is formed by the new species described here called *C. naaxtututs* and *C. tooteic*. There are previous records of the edibility of species of the genus *Calostoma*, including *C. cf. fuscum* in Malaysia (Abdullah & Rusea 2009), *C. junghuhnia* (Bandoni *et al.*1998) in Thailand and *C. cinnabarinum* in Mexico (Bautista-Nava & Moreno-Fuentes 2009). The new species described were collected exclusively at elevations ranging from 2,390 to 2,470 m in the soil of forests associated with *Quercus obtusata.* The present study contributes to the documentation of the traditional knowledge of the *Ayuuk jä'äy* culture, which has been conserved for centuries and is currently subject to accelerated cultural erosion.

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