Genotoxic Profile and Morphological Variation of the *Amanita rubescens* Complex: Traditional Knowledge for Safe Consumption in Mexico

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Abstract Wild mushrooms are important to the nutritional health and economic subsistence of rural populations in Mexico, but inaccurate identification of mushrooms has led to reported cases of poisoning. The aim of this study is to establish genotoxic profiles of mushrooms of the putative *Amanita rubescens* complex and to link those profiles with morphological attributes that suggest a correct identification of mushrooms, in order to prevent poisoning. Several combinations of amplification products (AMA, PHA, POP1, and POP2 genes) were identified in *A. rubescens* fungi sold in traditional markets; these genes are related to the presence of toxic polypeptides and its enzymatic regulators. The sequences correspond to a previously reported toxic gene family (MSDIM). All samples with the complete toxic gene profile presented reddish to dark-brown sporomes; this is the only attribute that visually distinguishes samples with toxic potential. Our results suggest that the mushrooms sold in traditional Mexican markets do not correspond to the *A. rubescens* complex. We conclude that morphological variability allows for identification of edible and inedible mushrooms.

Received February 7, 2018
Accepted May 23, 2019
Published September 4, 2019

Keywords Ethnomycology, Genotoxic profile, Edible mushrooms, α-Amanitin, Phallacidin

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Supplementary Files available at ojs.ethnobiology.org/index.php/ebl/article/view/1259

Introduction Mushrooms are outstanding for their diversity and traditional uses that provide economic, cultural, and nutritional benefits. As a result, there is abundant traditional knowledge concerning mushrooms’ nomenclature, ecology, management, use, conservation, and identification (Bandala et al. 2014; Gry and Anderson 2014; Mariaca-Méndez et al. 2001). Mushroom consumption in Mexico is regionally variable with preferences for some local species that are found in traditional markets: *Amanita aff. caesarea*, *A. rubescens*, *Boletus edulis*, *Cantharellus cibarius*, *Lactarius indigo*, *Morchella esculenta*, *Ramaria* sp., *Russula brevipes*, and *Ustilago maydis*, among others (Bandala et al. 1997; Estrada-Martínez et al. 2009; Herrera and Guzmán 1961; Mariaca-Méndez et al. 2001; Montoya et al. 2003, 2014).

Traditional collectors’ criteria for identification of edible mushrooms are based on form, color, consistency, habitat, developmental stage, and season (Guzmán 1999; Hernández-Rico 2011; Hung et al. 2015; Jiménez-González et al. 2013; Montoya et al. 2003; Romero-Bautista 2007). However, there have been reported cases of poisoning associated with misidentification by inexpert consumers, who possess inaccurate or insufficient knowledge (Hernández-Rico 2011). In general, identification criteria to distinguish edible from poisonous species seem to rest not on detailed recognition of the second set but precise knowledge of the first (Ruan-Soto 2018).

Some authors suggest that edible mushrooms in the *Amanita rubescens* complex in North America...
constitute different taxa than the European species or cryptic (morphologically similar but genetically distinct) species (Tulloss and Lindgren 1994), which probably have different toxic profiles. Most of the toxins in *Amanita* have been studied and described, with α-amanitin (blocks protein synthesis) and phallacidin (hepato- and nephrotoxic), which are both in the MSDIM toxic gene family (Anderl et al. 2012), as identified by their effects and general location at the genus level. However, it is unknown whether the edible amanitas, such as those belonging to the *A. rubescens* complex, have genes associated with the expression of α-amanitin and phallacidin but less toxic allelic variants that make them safe to consume after cooking. In addition, the activation of toxic genes requires the presence of enzymatic regulators (POP1 and POP2) that transform the protoxin into its active form (Luo et al. 2010).

Genetic variation, including toxic genes, usually causes phenotypic changes that can be appreciated at a glance; identifying morphological features that allow the discrimination of toxic and non-toxic genotypes should help to prevent poisoning (Anderl et al. 2012; Cai et al. 2014; Ferrerino et al. 2013; Hallen et al. 2007; Kendrick 2000; Lima et al. 2012; Luo et al. 2010). The aim of this research was to identify the presence of genes associated with toxicity in mushrooms of the *A. rubescens* complex sold for human consumption and to associate genotoxic profiles with morphological variation to establish a putative diagnostic attribute that can be used for safe consumption.

**Materials and Methods**

**Sampling**

Thirty sporocarps (i.e., the fruiting body of the fungi) of *A. rubescens* specimens were collected from five regions of Hidalgo, Mexico (Acaxochitlan, Huasca, Mineral del Chico, Pachuca, and Omitlan), which include places with and without traditional consumption of these mushrooms (Table 1; Figure 1). The samples were described, and locality, associated vegetation type, and other data were recorded. The biological material was kept in the mushroom collection of the Autonomous National University of Mexico (UNAM).

**DNA Extraction**

The standardized system of phenol-chloroform extraction, based on a modification of Gardes and Bruns' (1993) method, was used. Dry samples were processed in a laminar flow hood. Ammonium tissue fragment was put in a 1.5 µl microcentrifuge tube, submerged in liquid nitrogen, and macerated with a microbiological handgrip inside the tube. The macerate was suspended in 800 ml of CTAB 2x and incubated in a water bath at 65°C for one hour, moving the tubes every 20 minutes. Six hundred ml of chloroform-isoamyl alcohol (24:1) was added, mixed, and centrifuged at 13,000 rpm for ten minutes. Cold isopropanol (0.6 x) was added to the supernatant, mixed gently for a minute, and centrifuged as above. The pellet was cleaned with 500 ml 70% ethanol, dried, resuspended in 60 ml of dH2O, and stored at -20°C. The quality and concentration of DNA was assayed by spectrophometry (Bio Spectrometer Basic®).

**Amplification**

Markers specific to α-amanitin (Walton et al. 2004), phallacidin (Hallen et al. 2007), and two regulatory genes named POP (Luo et al. 2010) were used. As positive controls of the amplification, ITS1F, ITS4, and β-tubulin markers were used (White et al. 1990). Total reaction volume was 15 ml, consisting of 8.84 ml of dH2O, 2.5 ml of 10 x buffer (200 mm Tris–HCl, pH 8.4, KCl 500 mM), 0.16 ml of dNTPs (2 mM), 0.3 ml of MgCl2 (50 mM), 1 ml of each primer (50 mM), 0.75 U of Taq polymerase (Promega®), and 1 ml of DNA sample. The amplification conditions were 94°C for 8 minutes of initial denaturation, 35 cycles at 94°C for 30 seconds, 57°C for 30 seconds, 72°C for 1 minute, and 72°C for final amplification. The product evaluation was made by electrophoresis on acrylamide gels at 15%, over 50 minutes at 90 V. PCR products

### Table 1: Sample sites description and its traditional use of the *A. rubescens* complex.

| Municipality     | Locality                        | Vegetation | Edible | Common name                  |
|------------------|---------------------------------|------------|--------|------------------------------|
| Acaxochitlan     | La Montaña Viviente and Las Terrazas | Pine-oak   | No     | Crazy fungus                |
| Huasca           | Cerro del Zembo                 | Oak        | Sometimes | Unnamed                     |
| Mineral del Chico| Mineral del Chico               | Cedar      | No     | Unnamed                     |
| Pachuca          | San Miguel Cerezo market        | Pine-oak   | Yes    | Chiquita brisket or small cake|
| Omitlan          | Omitlan                         | Oak        | Yes    | Chiquita brisket            |
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were used for two subsequent rounds of nested PCR with the primers proposed by Hallen et al. (2007; Supplementary Table 1). Final PCR products of the six samples with a complete genotoxic profile and two probed toxic fungi (*Amanita* aff. *verna* and *A*. aff. *virosa*) were sequenced (Macrogen®), aligned (MEGA 7.0.26; Kumar et al. 2015), and compared with the GenBank database (BLAST-NCBI).

**Morphological Analysis**

Macro- and micromorphological attributes were described, with continuous (data that can take any value) and discontinuous (variation that can fall into a number of categories or classes) morphological data separated (Table 2). The macromorphological data were 1) pileus (cap): form, color, ornamentation, color of ornamentation, texture (discontinuous data), thickness of the context, diameter, and number of grooves (continuous data); 2) stipe (stem): form, color, ornamentation, color of ornamentation, texture (discontinuous data), thickness of the context, and length and width of the stipe (continuous data); 3) lamella (gills): color, frequency, and edge type (discontinuous data); and 4) ring: form, color, and position (discontinuous data). The micromorphological data were: 1) Melzer’s reaction (amyloid or inamyloid); 2) spore size (length and width); and 3) basidia size (length and width), which are attributes for taxonomic description of mushrooms according to Largent and Baroni (1988).

**Analysis**

Continuous morphological attributes were used to group the samples based on Ward’s method of amalgamation with Euclidean distances. Discontinuous morphological attributes were grouped by simple linkage with Gower’s distances, specific to the diverse nature of attributes. The genetic matrix with a specific amplification of genes related to toxicological profile was grouped by UPGMA (unweighted pair group method with arithmetic mean) clustering based on Jaccard’s distances (amplified presence-absence data set; Lloyd 2016). For all generated trees, the standard number of groups was obtained by the bootstrap method at 10,000 steps. Paired distance matrix (genetic with Jaccard’s distances, morphological by Euclidean distance for continuous data and Gower’s distances for discontinuous data) were correlated with a Mantel test to establish a correspondence between the genetic profile and morphological traits, following

**Figure 1** Geographic locations of the sampling sites of *A. rubescens* complex mushrooms.
transformation of the data with the formula z = (x - μ)/σ, where x is the original distance between individual, μ is the average distance, and σ is the deviation of the distance. This transformation was used to orthogonalize the magnitude of the distances.

**Results**

Molecular analysis grouped the mushrooms in two clades and one isolated individual from Pachuca de Soto with ITS1F as the unique amplified gene (Figure 2a). Individuals in the first group mostly amplified only to the control genes (seven individuals in black ¥), while some individuals presented one or two toxic profile genes (three individuals in orange †). The second group consisted of six individuals from the Pachuca market that amplified to all toxic profile genes (three individuals in orange †). The second amplified sequence of the red group of *A. rubescens* corresponds to *A. pallidoresea* α-amanitin gene cds (84%, KC778580.1), *A. exitialis* α-amanitin gene cds (71%, KC778563.1), and *A. fulginea* α-amanitin gene cds (71%, KC778575.1). Positive controls (4. aff. *verna* and 4. aff. *virosa* respectively) corresponded to the α-amanitin gene from *A. pallidoresea* (84% and 82% identity, KC778580.1), *A. phalloides* (both 79%, KC778577.1), *A. fulginea* (78% and 79%, F555208.1), and *A. exitabilis* (68% and 79%, KF813063.1).

The second amplified sequence of the red group corresponds to phallacidin (PHA1) partial genes from *A. biosporigera* (identity 86%, EU196141.1), *A. virosa* (84% FN555144.1), and *A. exitialis* (84% KC778564.1). Controls (4. aff. *verna* and 4. aff. *virosa* respectively) correspond to partial phallacidin genes from *A. biosporigera* (82% and 81%, EU196143.1), *A. virosa* (80% and 79%, FN555144.1), and *A. exitabilis* (78% and 76%, KF813064.1), all of them poisonous.

The grouping by simple linkage with Gower’s distances showed four groups (Figure 2b). All individuals with the complete genotoxic profile are included in the second group. The distinctive feature of the group was the reddish color of the stipe

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**Table 2** Categorical traits of the groups formed by the simple linkage agglomerative method of Mexican *A. rubescens* complex. All genotoxic profiles are included in the group 2.

| Categorical Trait                          | Group 1                  | Group 2                  | Group 3                  | Group 4                  |
|-------------------------------------------|--------------------------|--------------------------|--------------------------|--------------------------|
| Pileus color                              | Brown                    | Reddish-brown            | Brown                    | Brown                    |
| Pileus form                               | Convex                   | Convex                   | Convex                   | Depressed                |
| Pileus ornamentation                      | Fibrillas                | Verruca                  | Verruca                  | Without ornamentation    |
| Color of the pileus ornamentation         | Reddish-brown            | Reddish-brown            | Darkbrown                | Without ornamentation    |
| Color of the pileus context               | Pink                     | Beige                    | Beige                    | Pink                     |
| Texture of the pileus context             | Spongy                   | Fleshy                   | Fleshy                   | Spongy                   |
| Stipe color                              | Pink                     | Cream                    | Cream                    | Pink                     |
| Stipe form                               | Claviform                | Cylindrical              | Claviform                | Cylindrical              |
| Stipe ornamentation                       | Fibrillas                | Fibrillas                | Fibrillas                | Fibrillas                |
| Color of the stipe ornamentation          | Pink                     | Reddish*                 | Pink                     | Pink                     |
| Color of the stipe context                | Without color            | Pink                     | Pink                     | Pink                     |
| Texture of the stipe context              | Fibrous                  | Spongy                   | Spongy                   | Fibrous                  |
| Lamella color                            | Beige                    | Cream                    | Cream                    | Cream                    |
| Frequency of the sheets                   | Close together           | Close together           | Close together           | Close together           |
| Edge of thelamella                        | Sawing                   | Fimbriated               | Fimbriated               | Fimbriated               |
| Ring form                                | Fragile                  | Membranous               | Membranous               | Membranous               |
| Ring color                               | Beige                    | Cream                    | Cream                    | Beige                    |
| Ring position                            | Subapical                | Apical                   | Subapical                | Apical                   |
| Spores form                              | Ellipsoid                | Ellipsoid                | Ellipsoid                | Ellipsoid                |
| Spores staining (Melzer’s reaction)       | Amyloid                  | Amyloid                  | Inamyloid                | Amyloid                  |

*Exclusive characteristic of Group 2.
Figure 2 Geographic A Molecular grouping using the UPGMA method, B simple linkage grouping with Gower’s distance of morphological discontinuous data, and C ward grouping with Euclidian distances of continuous morphological variables. Red samples (‡) showed all molecular amplifies including genotoxic and positive controls of A. rubescens complex in Hidalgo, orange samples (†) showed one to three genotoxic markers, black samples (¥) only showed positive control amplifies.
ornamentation. In general, all individuals with complete or partial genotoxic profile had a darker reddish coloration (Table 2; Figure 3).

The Ward’s grouping showed three morphological groups associated with sporome size (Figure 2c). The smaller mushrooms are grouped in the third group, although they have wide stretch marks on the pileus; the largest mushrooms are grouped in the second group, and do not have stretch marks on the pileus. The first group is made up by medium size mushrooms (Table 3). However, none of these morphological groups are related to the genotoxic

Table 3 Description of the groups formed with Euclidean distances obtained from continuous traits of the *A. rubescens* complex.

|                      | Group 1          | Group 2          | Group 3          |
|----------------------|------------------|------------------|------------------|
| **Macromorphology**  |                  |                  |                  |
| Minimum diameter of  | 48.2 ± 16.22     | 78 ± 24.97       | 6.94 ± 20.2      |
| the pileus (mm)      |                  |                  |                  |
| Maximum diameter of  | 81.8 ± 27.2      | 102.5 ± 19.89    | 80.67 ± 23.1     |
| the pileus (mm)      |                  |                  |                  |
| Stretch length (mm)  | 0.8 ± 2.04       | 0                | 1.06 ± 1.9       |
| Context of the pileus| 6 ± 1.79         | 5.67 ± 0.52      | 6.11 ± 3.4       |
| (mm)                 |                  |                  |                  |
| Minimum longitude of | 91.5 ± 39.24     | 0                | 0                |
| the stipe (mm)       |                  |                  |                  |
| Maximum longitude of | 114.2 ± 32.31    | 92.5 ± 18.06     | 70.5 ± 42.1      |
| the stipe (mm)       |                  |                  |                  |
| Minimum width of the | 13.2 ± 3.71      | 0                | 0.72 ± 3.1       |
| stipe (mm)           |                  |                  |                  |
| Maximum width of the | 13.8 ± 10.09     | 14 ± 2.1         | 13.22 ± 5        |
| stipe (mm)           |                  |                  |                  |
| Context of the stipe | 16 ± 4.69        | 14 ± 2.1         | 13.22 ± 5        |
| (mm)                 |                  |                  |                  |
| **Micromorphology**  |                  |                  |                  |
| Minimum longitude of | 7.4 ± 0.92       | 7.33 ± 0.5       | 7.4 ± 0.5        |
| the spore (µ)        |                  |                  |                  |
| Maximum longitude of | 10.4 ± 1.07      | 9.73 ± 0.25      | 10.43 ± 1        |
| the spore (µ)        |                  |                  |                  |
| Minimum width of the | 5.9 ± 0.46       | 5.72 ± 0.44      | 5.64 ± 0.5       |
| spore (µ)            |                  |                  |                  |
| Maximum width of the | 8 ± 0.84         | 7.92 ± 0.48      | 7.81 ± 0.8       |
| spore (µ)            |                  |                  |                  |
| Minimum longitude of | 25.7 ± 2.31      | 22.57 ± 1.76     | 21.22 ± 6        |
| the basidia (µ)      |                  |                  |                  |
| Maximum longitude of | 35.3 ± 2.55      | 32.87 ± 2.37     | 31.16 ± 8.7      |
| the basidia (µ)      |                  |                  |                  |
| Minimum width of the | 9 ± 0.49         | 7.58 ± 1.45      | 7.27 ± 2         |
| basidia (µ)          |                  |                  |                  |
| Maximum width of the | 11.7 ± 0.84      | 10.28 ± 1.32     | 10.07 ± 2.7      |
| basidia (µ)          |                  |                  |                  |

*Figure 3* Samples of *A. rubescens* complex with reddish color detail. **A** GNHR-1 (AMA, PHA positive), **B** GNHR-4 (AMA, PHA positive), **C** GNHR-9 (complete genotoxic profile), **D** GNHR-16 (only POP1 negative), **E** GNHR-18 (only PHA negative), and **F** GNHR-29 (no toxic profile). The reddish color of the sample with the complete profile is highlighted (c), the brown color of the samples with a partial genotoxic profile (a, b, d, e) and the light color of the negative sample (f).
thermolabile toxin called rubescenslysin, which interacts with phospholipids in cell membranes, generating intracellular hemolysis, cardiotoxicity, and adverse effects in the central nervous system. The sequence of genes associated with this toxin is not yet known, so no appropriate molecular markers are available (Odenthal et al. 1982; Seeger and Wachter 1980).

With respect to the categorical traits associated with the presence of AMA, PHA, POP1, and POP2 genes; the distinctive attribute for recognizing a potentially toxic mushroom is the dark reddish stipe ornamentation (Table 2; Figure 2b). The other five recorded traits are shared between groups that present partial genotoxic profiles. However, in general, it could be said that more intense reddish-brown-colored mushrooms tend to have more genes associated with toxicity.

The presence of genes responsible for the expression of toxins in the *A. rubescens* complex suggests that they are potentially dangerous; the complex is accordingly assigned to section Phalloidae, where most species are toxic (Cai et al. 2014). The variability of combinations in genotoxic profiles could relate to deletions and/or duplications in the copy number of the sequences AMA and PHA, which give rise to hypervariable regions that code for different peptides of between seven to ten amino acids (Hallen et al. 2007). However, we cannot assume that the fungi really are toxic, as it is unknown which genes are expressed and to what extent. To elucidate toxicity, assays by liquid chromatography mass-spectrometry (Parmen et al. 2016) for the detection of expression products are necessary.

The fact that the AMA and PHA genes are absent in non-toxic fungi in section Phalloidea (Hallen et al. 2007) suggests that the mushrooms consumed in Hidalgo are mistakenly identified as species within the *A. rubescens* complex, or that some edible non-toxic mushrooms in section Phalloidea conserve the potential to express toxicity. In addition, fungi can have other compounds that result in poisoning in combination with alcoholic beverages or some foods. For example, *Coprinus atramentarius* has coprine, which in combination with alcohol causes pain and sickness (Graeme 2014; Gry and Andersoon 2014; Jo et al. 2014). In particular, *A. rubescens* presents a thermolabile toxin called rubescenslysin, which interacts with phospholipids in cell membranes, generating intracellular hemolysis, cardiotoxicity, and adverse effects in the central nervous system. The sequence of genes associated with this toxin is not yet known, so no appropriate molecular markers are available (Odenthal et al. 1982; Seeger and Wachter 1980).

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On the other hand, morphological variation of the continuous characteristics did not show any structure that would allow for the identification of toxicity. Traditional sellers group mushroom according to size for pricing; attributes such as size, width, and weight that are traditional criteria of the vendors do not appear to be related to toxicity (Burrola-Aguilar et al. 2012; Hernández-Rico 2011; Rodríguez-Muñoz et al. 2012).

**Conclusions**

Women are the main fungal collectors in the central mountainous region of Hidalgo. In general, the criteria for choosing edible fungi vary across regions, so some cases of mycetism in Acaxochitlan and Real del Monte have removed *A. rubescens* from local diet. Our results suggest modifying the traditional criteria for the selection of edible fungi, those similar to *A. rubescens*, by considering the color intensity. In addition, the possibility of horizontal transfer of MSDIM genes and/or the preservation of toxic potential in the genome of *A. rubescens* (from Hidalgo, at least), brings into question the viability of continuing consume this fungus.

In conclusion, these results show that a macromorphological characteristic, color, is associated with a complete or partial genotoxic profile among edible fungi identified as *A. rubescens*. This is not yet a solid guideline for the collection of guaranteed edible mushrooms. In order to develop more conclusive recommendations for traditional mushroom collectors and vendors, these results must be replicated using a larger sample size, and the evaluation of toxicity must
considerer traditional collectors’ criteria used to form morphological groups. However, these results are a significant contribution to developing restrictive criteria (intense reddish-brown-colored mushrooms) for the avoidance of potentially toxic fungi.

Acknowledgments
We thank the women of the high region of Hidalgo, who allowed us to live together and learn from their traditional knowledge. We also thank CONACYT for the grant to the first author and INFR-252807 for financial support.

Declarations
Permissions: None declared.
Sources of funding: CONACYT grant to GNHR and INFR-252807 project.
Conflicts of Interest: None declared.

References Cited
Anderl, J., H. Echner, and H. Faulstich. 2012. Chemical Modification Allows Phallotoxins and Amatoxins to be Used as Tools in Cell Biology. Beilstein Journal of Organic Chemistry 8:2072–2084. DOI:10.3762/bjoc.8.233.

Bandala, V. M., L. Montoya, and I. Chapela. 1997. Wild Edible Mushrooms in Mexico: A Challenge and Opportunity for Sustainable Development. In Mycology in Sustainable Development: Expanding Concepts, Vanishing Borders, edited by M. Palm, and I. Chapela, pp. 76–90. Parkway Publishers, Boone, NC.

Bandala, V. M., L. Montoya, R. Villegas, T. G. Cabrera, M. J. Gutiérrez, and T. Acero. 2014. “Nangañaña” (Tremelloscypha gelatinosa, Sebacinaeae), Hongo Silvestre Comestible del Bosque Tropical Deciduo en la Depresión Central de Chiapas, México. Acta Botánica Mexicana 106:149–159. DOI:10.21829/abm106.2014.216.

Burróla-Aguilar, C., O. Montiel, R. Garibay-Orjiel, and L. Zizumbo-Villareal. 2012. Conocimiento Tradicional y Aprovechamiento de los Hongos Comestibles Silvestres en la Región de Amanalco, Estado de México. Revista Mexicana de Micología 35:1–16.

Cai, Q., R. E. Tulloss, L. P. Tang, B. Tolgor, P. Zhang, Z. H. Chen, and Z. L. Yang. 2014. Multi-locus Phylogeny of Lethal Amanitas: Implications for Species Diversity and Historical Biogeography.
Hunn, E. S., Y. Venegas-Ramírez, and M. A. Vázquez-Dávila. 2015. Where Do Fungi Fit? The Fungal Domain in Mixtepec-Zapotec. *Journal of Ethnobiology* 35:286–313. DOI:10.2993/etbi-35-02-286-313.1.

Jiménez-González, M., L. Romero-Bautista, M. A. Villavicencio-Nieto, and B. E. Pérez-Escandón. 2013. Los Hongos Comestibles de la Región de Molango de Escamilla, Hidalgo, México. In *Estudios Científicos en el Estado de Hidalgo y Zonas Aledañas, Vol II*, edited by G. Pulido-Flores and S. Monks, pp. 69–82. Universidad Autónoma del Estado de Hidalgo Press, Mineral de la Reforma, Hidalgo, Mexico.

Jo, W., A. Md. Hossain, and S. Park. 2014. Toxicological Profiles of Poisonous Edible and Medicinal Mushrooms. *Mycobiology* 42:215–220. DOI:10.5941/MYCO.2014.42.3.3215.

Kendrick, B. 2000. *The Fifth Kingdom*. Focus Publishing, Newburyport, MA.

Kumar, S., G. Stecher, and K. Tamura 2015. MEGA7: Molecular Evolutionary Genetic Analysis, Version 7.0.26. Available at: http://www.kumarlab.net/publications. Accessed on December 5, 2017.

Largent, D. L., and T. J. Baroni. 1988. *How to Identify Mushrooms to Genus VI: Modern Genera*. Mad River Press, Eureka, CA.

Lima, A. D. L., R. Costa Flores, M. R. C. Garbinoaes, and S. Percário. 2012. Poisonous Mushrooms: A Review of the Most Common Intoxications. *Nutrición Hospitalaria* 27:402–408. DOI:10.1590/S0212-16112012000200009.

Lloyd, G. T. 2016. Estimating Morphological Diversity and Tempo with Discrete Character-Taxon Matrices: Implementation, Challenges, Progress, and Future Directions. *Biological Journal of the Linnean Society* 118:131–151. DOI:10.1111/bij.12746.

Luo, H., H. E. Hallen-Adams, J. S. Scott-Craig, and J. D. Walton. 2010. Colocalization of Amanitin and Candidate Toxin-processing Prolylloigio Peptidase in *Amanita Basidiocarps*. *Eukaryotic Cell* 9:1891–1900. DOI:10.1128/EC.00161-10.

Mariaca-Méndez R., L. Del C. Silva-Pérez, and C. A. Castarios-Montes. 2001. Proceso de Recolección y Comercialización de Hongos Comestibles Silvestres en el Valle de Toluca, México. *Ciencia Ergo Sum* 8:30–40. Available at: http://www.redalyc.org/articulo.oa?id=10402004. Accessed on December 7, 2017.

Montoya, A., O. Hernández-Totomoch, A. Estrada-Torres, A. Kong, and J. Caballero. 2003. Traditional Knowledge About Mushrooms in a Nahua Community in the State of Tlaxcala, Mexico. *Mycologia* 95:793–806. DOI:10.1080/15572536.2004.11183308.

Montoya, A., A. Kong, R. Garibay-Orijel, C. Méndez-Espinoza, R. E. Tulloss, and A. Estrada-Torres. 2014. Availability of Wild Edible Fungi in La Malinche National Park, México. *Journal of Mycology* 241806. DOI:10.1155/2014/241806.

Odenthal, K. P., R. Seeger, R. Braatz, E. Petzinger, H. Moshaf, and C. Schmitz-Drager. 1982. Damage in Vitro to Various Organs and Tissues by Rubescenslysins from the Edible Mushroom *Amanita rubescens*. *Toxicon* 20:765–781. DOI:10.1016/0041-0101(82)90124-6.

Parnmen, S., S. Sikaphan, S. Leudang, T. Boonpratuang, A. Rangsiriruj, and K. Naksuwankul. 2016. Molecular Identification of Poisonous Mushrooms Using Nuclear ITS Regions and Peptide Toxins: A Retrospective Study on Fatal Cases in Thailand. *Journal of Toxicology Science* 41:65–76. DOI:10.2131/jts.41.65.

Rodríguez-Muñoz, G., E. Zapata-Martelo, M. N. Rodríguez, V. Vázquez-García, B. Martínez-Corona, and I. Vizcarra-Bordi. 2012. Saberes Tradicionales, Acceso, Uso y Transformación de Hongos Silvestres Comestibles en Santa Catarina del Monte, Estado de México. *Agricultura, Sociedad y Desarrollo* 9:191–207.

Romero-Bautista, L. 2007. La Sistemática: Base del Conocimiento de la Biodiversidad. In *Avances en la Taxonomía y Sistemática de los Hongos: Una Revisión General*, edited by A. Contreras Ramos, C. Cuevas Cardona, I. Goyenechea, and U. Iturbe, pp. 67–74. Universidad Autónoma del Estado de Hidalgo Press, Mineral de la Reforma, Hidalgo, Mexico.

Ruan-Soto, F. 2018. Sociodemographic Differences in the Cultural Significance of Edible and Toxic Mushrooms Among Tsotsil Towns in the Highlands of Chiapas, Mexico. *Journal of Ethnobiology and Ethnomedicine* 14:32. DOI:10.1186/s13002-018-0232-9.
Segeer, R., and B. Wachter. 1980. Rubescenslysin and Phallolysin Release Marker Molecules from Phospholipids Cholesterol Liposomes. *Acta Biochemistry and Biophysics* 645:59–62.

Tullos, R. R., and J. E. Lindgreen. 1994. *Amanita novinupta-α*—A Rubescent, White Species from the Western United States and Southwestern Canada. *Mycotaxon* 51:179–190.

Walton, J. D., D. G. Panaccione, and H. E. Hallen. 2004. Peptide Synthesis without Ribosomes. In *Advances in Fungal Biotechnology for Industry, Agriculture and Medicine*, edited by J. Tkacz and L. Lange, pp. 127–162. Kluwer Academic, New York.

White, T. J., T. Bruns, S. Lee, and J. Taylor. 1990. Amplification and Direct Sequencing of Fungal Ribosomal RNA Genes for Phylogenetics. In *PCR Protocols: A Guide to Methods and Applications*, edited by M. A. Innis, D. H. Gelfand, J. J. Sninsky, and T. J. White, pp. 315–322. Academic Press, New York.