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Morphological characteristics of wild and cultivated *Ganoderma subincrustatum* from Sonora, Mexico

Características morfológicas de *Ganoderma subincrustatum* silvestre y cultivada de Sonora, México

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RESUMEN

Antecedentes: *Ganoderma* es un género complejo con alta variabilidad morfológica. *Ganoderma subincrustatum* es una especie parásita y saprobia común y su circunscripción es dudosa para algunos autores. El objetivo de este trabajo fue analizar la variabilidad morfológica de *G. subincrustatum* silvestre y cultivada.

Métodos: La cepa de *Ganoderma subincrustatum* se aisló de una huerta de durazno en La Costa de Hermosillo, Sonora. La cepa se cultivó sobre residuos de poda de vid bajo dos condiciones de iluminación baja y alta. Los basidiomas silvestres y cultivados se caracterizaron y compararon macro y microscópicamente.

Resultados y conclusiones: *Ganoderma subincrustatum* se registra por primera vez para la micobiota Sonorense. La forma, tamaño y color entre basidiomas silvestres y cultivados con iluminación baja y alta fueron diferentes; esta última causó forma de asta de los cuerpos fructíferos. Los especímenes silvestres y cultivados mostraron el mismo tipo de contexto, forma similar de células de la cutícula y basidiosporas, pero basidiosporas más largas en los silvestres.

Palabras clave: taxonomía, circunscripción, tipo de contexto, células de la cutícula

ABSTRACT

Background: *Ganoderma* is a complex genus with high morphological variability. *Ganoderma subincrustatum* is a common parasite and saprophytic species, and its circumscription is doubtful for some authors. The aim of this study was to analyze the morphological variability of wild and cultivated specimens of *G. subincrustatum*.

Methods: *Ganoderma subincrustatum* strain was isolated from a peach orchard in La Costa de Hermosillo, Sonora. The strain was cultivated on vineyard pruning wastes under low and high illumination. Wild and cultivated basidiomata were macro and microscopically characterized and compared.

Results and conclusions: *Ganoderma subincrustatum* was recorded for the first time from Sonoran mycobiota. Basidiomata color, size and shape were different between wild and cultivated under low and high illumination. High lux condition caused antler shape fruiting bodies. Wild and cultivated specimens showed the same type of context, similar shape of pileipellis cells and basidiospores, but basidiospores were larger in wild specimens.

Keywords: taxonomy, circumscription, context type, pileipellis cells

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INTRODUCTION

Ganoderma P. Karst. (Polyporales: Ganodermataceae) is a genus with cosmopolitan distribution, registered from tropical, temperate, agricultural and desert-like areas (Gottlieb and Wright 1999; Torres-Torres *et al.*, 2012; López-Peña *et al.*, 2016). Morphological plasticity makes *Ganoderma* one of the most complex genus in Polyporales; a sign of this are the 290 published names, many of these are considered synonyms (Ryvarden, 2000). Therefore, different molecular markers for species delimitation have been assessed (Douanla-Meli and Langer, 2009; Wang *et al.*, 2012).

The taxonomy of *Ganoderma* is usually based on macro and micromorphological characters, being the most important shape and size of basidiospores and pilei-

pellis cells (Steyaert, 1972; Gottlieb and Wright, 1999; Ryvarden, 2000). Recently, resinous incrustations and context type were used to discriminate some species, since they also are constant characters (Torres-Torres *et al.*, 2012). A not fully homogeneous context with resinous bands and incrustations are characteristics of *Ganoderma subincrustatum* Murrill, a species recorded from Argentina, Jamaica, and Mexico (Torres-Torres *et al.*, 2015).

Most of the farm products from La Costa de Hermosillo are produced to exportation purposes, and annually big losses are caused by *Ganoderma* infections in peach orchards. *G. subincrustatum* is a facultative parasite or saprobic species recorded mainly from tropical and subtropical areas, even in *Pinus-Quercus* forest (Torres-Torres *et al.*, 2015). There are few works based on fruiting bodies development under controlled conditions for biotechnological purposes such as improving the production of medicinal mushrooms (Islam *et al.*, 2011; Sudheer *et al.*, 2018).

To our knowledge, there are no studies on morphological characterization to determine the variability of main taxonomic characters between wild and cultivated *Ganoderma* species. Due to this fact, the aim of this study was to compare the morphological characteristics of wild and cultivated specimens of a *G. subincrustatum* strain, and to evaluate the effect of culture conditions, mainly light intensity, on fruiting bodies development in order to investigate if taxonomic characters remain constant among them.

MATERIALS AND METHODS

Basidiomata and strain

Basidiomata were collected on a living *Prunus persica* (L.) Batsch tree in a peach orchard at La Costa de Hermosillo, Sonora, Mexico, September 7, 2012, and deposited in the Mushroom Collection of Universidad Estatal de Sonora under voucher UES (10500). On that date, temperature varied from 24 to 37 °C, and relative humidity from 24 to 89 % (Weather Underground, 2016). This orchard is under constant watering; thus, moisture in the environment remains high. Sections of hymenophore were placed in Petri dishes with malt extract agar (MEA), supplemented with chloramphenicol (0.5 gL^{-1}) and benomyl (22 mgL^{-1}). After mycelium development, sections were transferred to MEA without supplements. Mycelia-samples from this second culture were examined to find clamp connections (Largent *et al.*, 1977) with an Olympus BX51 microscope. Abundant clamps were observed in the isolated mycelia, which serve as indicative of good isolation, because clamps are basidiomycete's specific structures. Likewise, cultivated basidiomata were harvested for morphological characterization and deposited under vouchers UES (10501, 10502, 10503, 10504). *Ganoderma subincrustatum* strain was maintained on MEA Petri dishes.

Inoculum and cultivation conditions

Inoculum preparation was carried out using wheat grain. Grain was soaked for 24 h in distilled water to reach 60 % humidity, later sterilized at 121 °C for 1.5 h. After temperature was down, wheat grain was inoculated with portions of the MEA culture. Vineyard pruning wood chips unsupplemented (2-3 cm in length) were hydrated for 12 h, drained and sterilized in polypropylene bags (700 g of wet substrate per bag) for 1.5 h at 121 °C. The bags were inoculated with 5 % (w/w) of wheat grain spawn and incubated in darkness at 25±1 °C until substrates were fully colonized (15-17 d). Basidiomata development was performed under two conditions, one of them was maintained at 25±1 °C, 85-90 % of relative humidity (RH) and 350 lux (low light condition). The other was 27±1 °C, 90-100 % RH and 3500-4000 lux (high light condition), photoperiod for both treatments was 12 h (Stamets, 2000) using cold-light fluorescent lamps and CO_2 was maintained at 500-600 ppm, because is an important factor for fruiting bodies development. Basidiomata were harvest when growth stopped, with two developmental shapes: antler (3500-4000 lux treatment), and flabelliform fully developed pileus (350 lux treatment) to study macro and microscopic features (Gottlieb and Wright, 1999; Torres-Torres and Guzmán-Dávalos, 2012).

Morphological characterization

For morphological characterization, thin sections of basidiomata were mounted in 10 % KOH and analyzed using an Olympus BX51, with an Infinity Analyze 2 integrated camera for microscopic descriptions. Basidiospore shape was expressed according to Q ratio (length/width) of at least 20 randomly selected spores (Largent *et al.*, 1977). Color descriptions were made according to Kornerup and Wanscher (1978). Because *G. subincrustatum* is a first record from Sonora, a full description of wild basidiomata is presented.

RESULTS

Wild basidiomata description

Basidiomata 178-270 × 117-124 × 22-70 mm, substipitate to sessile, dimidiate, fibrous to spongy. Pileus flabelliform, surface glabrous, smooth, slightly dented and corrugated, bright to dull, with semi-concentric furrows, more conspicuous to the margin, cuticle light orange (5A5), reddish-golden (6C7), deep orange (6A8), light brown (6D8), reddish-brown (8E6), dark brown (8F8) to photo brown (9F8), almost black in some zones, with deep yellow (4A8) furrows due to cuticle detaching, covered by a brown (6E8) basidiospores layer; margin sterile, obtuse, smooth, with groove zones, pale yellow (4A3), with yellowish-orange (4A7) zones, greyish yellow (4C7) after contact. Context up to 22 mm thick, fibrous-spongy, not fully homogenous, with a deep yellow (4A8) band under the cuticle, light orange (5A5) to brown (5F7) toward the tubes, concentrically zonate, with two resinous bands, the upper thickened, both intermittent, ¾ context length, interrupted near the margin. Tubes up to 14 mm long, brown (5E8), orange white (5A2) towards the pores surface, unstratified. Pores 3-5 per mm, 133-171 µm diam, pale yellow (4A3), greyish-yellow (4C7) after contact, angular to rounded, with irregular edge. Stipe 50-75 × 29-52 mm, lateral, smooth, shiny to dull, photo brown (9F8), flattened to cylindrical, solid, fibrous to spongy, concentrically zonate.

Trimitic hyphal system. Generative hyphae 1.6-7.2 µm diam, septate, fibulate, hyaline to pale yellow, difficult to observe, generally collapsed; skeletal hyphae arboriform to non-branched, 1.6-7.2 µm diam, solid to thick-walled, light brown; connective hyphae branched, 1.6-3.2 µm diam, thick-walled, hyaline to pale yellow. Pileipellis as a crustohymeniderm, cells 31.2-90.4 × 8-12 µm, narrow clavate to clavate, commonly with one to three protuberance or branches, solid to tick walled, sometimes multistratified, pale yellow to deep yellow. Basidiospores (8-)9.6-11.2(-12.8) × 5.6-8 µm, Q= 1.49-1.64, ellipsoids to oblong, apex truncate, with apical germ pore, exosporium with subfree to partially anastomosed interwalled pillars, reddish-brown.

Wild *versus* cultivated basidiomata

Basidiomata variability in size, color and shape were observed among wild and cultivated specimens, also among those cultivated under different illumination treatments (Table 1, Figure 1). Context type remained similar between wild and cultivated basidiomata, even those harvested with antler-like shape, which remains as a significant character in *G. subincrustatum*. Shape of pileipellis cells and basidiospores seems to be a constant character in *G. subincrustatum*. In both cases, cells were clavate, with two or three lateral or apical branches or protuberances, light to deep yellow, some of them with apical incrustations.

Slight basidiospores variability between wild and cultivated specimens was observed. Wild basidiomata showed larger basidiospores, mostly ellipsoids, some oblong, while cultivated basidiomata mainly ellipsoids basidiospores, some broadly ellipsoids; all of them with subfree to partially anastomosed interwalled pillars.

Table 1. Morphological variability between wild and cultivated basidiomata of *Ganoderma subincrustatum*

	Wild	Cultivated 350 lux	Cultivated 3500-4000 lux
Basidiomata size	178–270 × 117–124 × 22–70 mm	59–84 × 39–75 × 6–19 mm	55–123 × 11–29 × 9–17 mm
Pileus shape	Flabelliform	Flabelliform	Antler
Context	up to 20 mm thick 2 resinous bands	up to 13 mm thick 2 resinous bands	up to 4 mm thick 1–2 resinous band
Hymenophore	Tubes up to 14 mm long	Tubes up to 8 mm long	undeveloped
Pileipellis cells	31.2–90.4 × 8–12 µm sometimes multistratified, with protuberances	42.3–58.8 × 5.3–9.6 µm with apical incrustations and protuberances	29–52.1 × 7.1–9.2 µm with apical incrustations and protuberances
Basidiospores	9.6–11.2 × 5.6–8 µm; Q= 1.49–1.64 ellipsoid to oblong	7.7–9.9 × 5.7–7.7 µm; Q= 1.21–1.47 ellipsoid	undeveloped

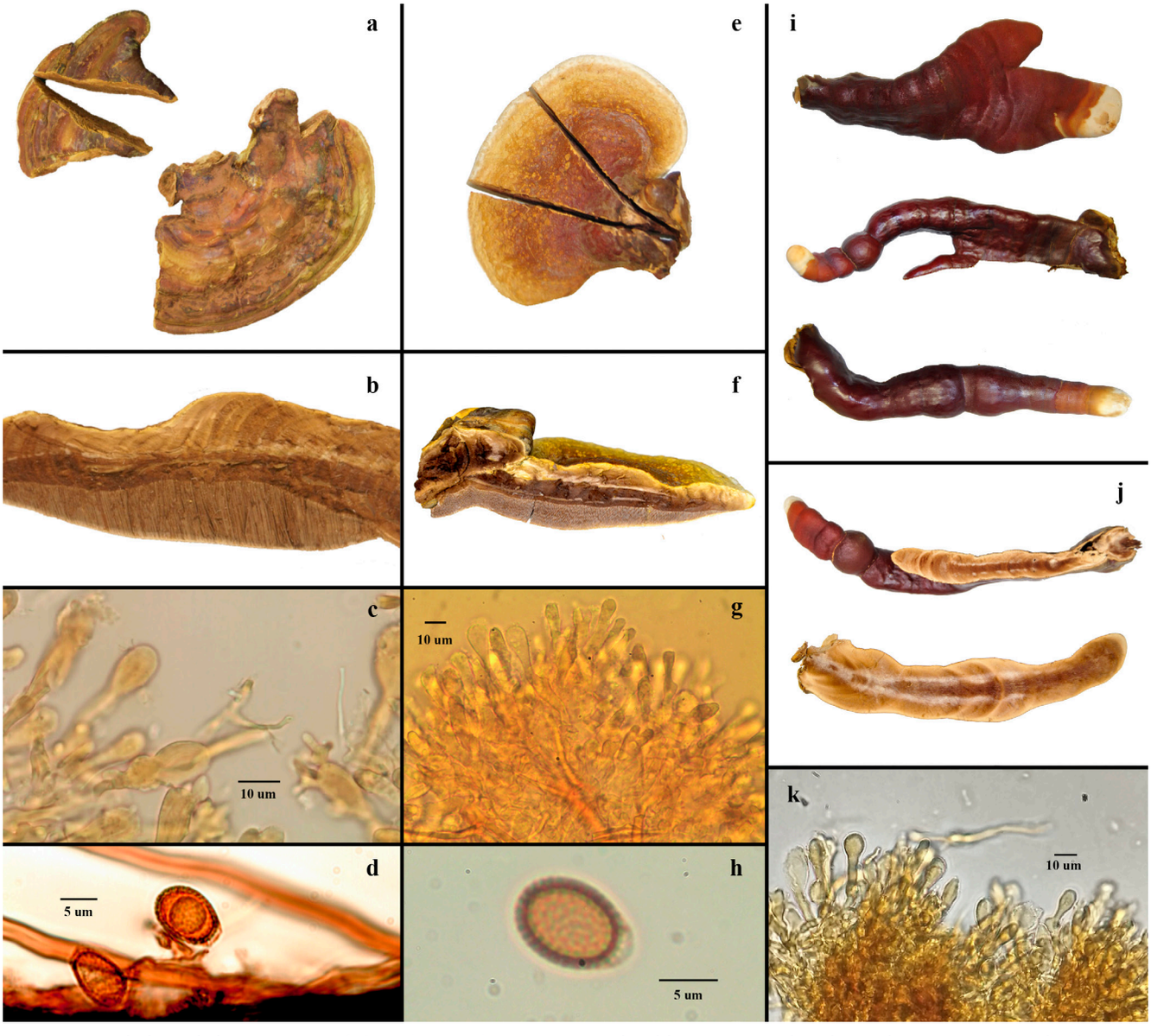

FIGURE 1. Morphological characteristics of *Ganoderma subincrustatum*. a-d: wild specimen. a: basidiome. b: context. c: cuticle elements. d: basidiospores. e-h: cultivated specimens under 350 lux. e: basidiomata. f: context. g: cuticle elements. h: basidiospore. i-k: cultivated specimens under 3500–4000 lux. i: basidiomata. j: context. k: cuticle elements.

DISCUSSION

Wild basidiomata presented a larger size than cultivated, which could be attributed to different environmental conditions. Some specimens under high light conditions were developed with antler-like shape, others with long stipe, and some tended to develop flabelliform pileus with hymenophore (Figure 1i). Stamets (2000) mentioned that under low light conditions, stipe elongation becomes slow and the mycelium enters into the pilei development period in *G. lucidum*, thus longer stipes in cultivated specimens could be attributed to illumination condition.

According with Torres-Torres *et al.* (2015), *G. subincrustatum* presents a perennial growth, but Gottlieb and Wright (1999) did not mention that character. In this study all basidiomata were harvested in their first developmental stage, even the wild specimen. Bazzalo and Wright (1982), and Ryvarden (2000) considered *G. subincrustatum* synonym under *G. resinaceum*, but Gottlieb and Wright (1999) contemplated it as independent species, with pileipellis cells of 33-45 × 8-10 µm, and basidiospores of (9-)10-12(-13)

× 6-9 µm. On the other hand, Torres-Torres *et al*. (2015) recognized *G. subincrustatum* as independent species, and report pilleipelis cells of 32-80(-96) × 5.5-14.5 µm, and basidiospores of 9.6-12.4 × 7.2-8.4 µm.

In the present work, we recorded smaller basidiospores than those characterized by Torres-Torres *et al*. (2015) for this species, which probably can be attributed to environmental conditions; however, the shape remains equal. Steyaert (1975) observed a positive influence of altitude or latitude on mean lengths of basidiospores. An increase in temperature seems to depress basidiospore size. Temperature varies in the same direction whether in altitude or latitude and would consequently be the ruling factor for mean basidiospores size.

Islam *et al*. (2011) worked with several light intensity (0-850 lux) in *G. lucidum* cultivation and development and reported the largest pileus diameter at 570 lux and the smallest at 0 lux. Sudheer *et al*. (2018) evaluated the CO_2 concentration and presence or absence of light (742 lux) on *Ganoderma* fruiting bodies development. They observed antler-like and spider-like fruiting bodies, depending on the presence or absence of light, respectively; both treatments with high CO_2 concentration. None of these studies evaluated light intensities higher than 850 lux. In our study, light intensity of 3500-4000 lux provoked antler-like fruiting bodies in *G. subincrustatum*. Although our treatments had a slight difference in temperature and RH, nevertheless, both were in the recommended range for production of *Ganoderma lucidum* basidiomata (Stamets, 2000; Lakshmi, 2013); thus, differences in basidiomata shape could be attributed to light intensity.

In summary, this study contributes to the circumscription of *G. subincrustatum*. Due to the intrinsic species variability recorded for *Ganoderma* spp. worldwide, it is necessary to continue working on its taxonomy, analyzing molecularly more species to understand the phylogeny of this genus, and to demonstrate *G. subincrustatum* is a real independent species.

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