

## Notes on *Cystangium pineti* and *Macowanites mexicanus* (*Russulaceae*)

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**Abstract** – *Cystangium pineti* is described and illustrated from the Transmexican Volcanic Belt. *Macowanites mexicanus* was found to be morphologically identical and is considered here as a syn. nov. for *C. pineti*. Morphological affinities with other known *Cystangium* as well as with agaricoid members of *Russula* are discussed.

### Mexico / sequestrate fungi / taxonomy

**Résumé** – *Cystangium pineti* est décrit et illustré de la Chaîne Volcanique Transmexicaine. *Macowanites mexicanus* s'est avéré morphologiquement identique et est considéré ici comme syn. nov. pour *C. pineti*. Les affinités morphologiques sont discutées non seulement par rapport aux autres *Cystangium* connus mais aussi par rapport aux espèces agaricoides du genre *Russula*.

## INTRODUCTION

*Cystangium* and *Macowanites* are two genera of sequestrate fungi that are phylogenetically related with *Russula* (Eberhardt & Verbeken, 2004; Miller *et al.*, 2007). Both have secotioid to gasteroid basidiomata. *Cystangium* is defined as having an epithelial to pseudoparenchymatic pileipellis whereas *Macowanites* has a pileipellis with a filamentous structure (Lebel & Trappe, 2000). Presence/absence of macrocystidia in the hymenium is also used to separate both genera (Singer & Smith, 1960). Poor knowledge of these two genera in Mexico may partly be due to their semihypogeous habit. Only three species have been described so far: (1) *Cystangium pineti* was described by Singer (1985) from one collection from the Cofre de Perote region, Veracruz, (2) *Macowanites mexicanus* was described by Guzmán (1988) and is based on material collected from the same area as *C. pineti*, but also from the Popocatepetl volcano, State of Mexico, and finally, (3) *Macowanites duranguensis*, equally described by Guzmán (1988) is based on a single collection made in La Michilía Biosphere Reserve, Durango. More recently, García *et al.* (1998) reported on the presence of *Gymnomyces*, *Macowanites*, and *Zelleromyces* in the state of Querétaro, but without any precision about the involved species. Finally, Calonge and Vidal (2005) described *Gastrolactarius denudatus*, a sequestrate *Russulaceae* with exposed hymenium exuding latex that

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was collected in the same Cofre de Perote region from which *C. pineti* and *M. mexicanus* were described.

During our mycological explorations in La Malinche National Park, Tlaxcala State, several specimens of sequestrate *Russulaceae* were collected on the slopes of the volcano. Some collections belong to *Cystangium* and others to *Macowanites*. The aim of this study is to present a critical morphological analysis of the specimens belonging in *Cystangium* and to compare these with described known secotioid and agaricoid taxa in *Russulaceae*.

## MATERIALS AND METHODS

Data on macroscopic features are based on original descriptions and field notes accompanying the studied specimens. Microscopic features were described from freehand sections of rehydrated specimens mounted in Melzer's reagent or 10% aqueous KOH. Terminology follows Buyck (1991) for the cells of the hymenium, and Heilmann-Clausen *et al.* (1998) for the type of pileipellis. Spore measurements within parentheses excluded height of ornamentation, and the latter is described and illustrated as observed in Melzer's reagent. Measurements are given as (MINa) [ $\underline{AVa} - 2*SD$ ] -  $\underline{AVa}$  -  $\underline{AVb}$  - [ $\underline{AVb} + 2*SD$ ] (MAXb) in which MINa = lowest value for the measured collections,  $\underline{AVa}$  = lowest mean value,  $\underline{AVb}$  = greatest mean value and SD = standard deviation, and MAXb = greatest value for the measured collections. Q stands for quotient length/width and is given in the same way as the other measurements. Mean values are underlined and are from at least 30 measurements of each structure. Abbreviation SV stands for sulfovanillin, LMNP for La Malinche National Park. Line drawings were made using a drawing tube. Basidia length excludes sterigmata length. Abbreviations of herbaria are according to Holmgren *et al.* (1990) or Holmgren and Holmgren (1995).

## RESULTS

*Cystangium pineti* Singer 1985, *Mycol. Helv.* 1(6): 417. Fig. 1-10  
= *Macowanites mexicanus* Guzmán 1988, *Rev. Mex. Mic.* 4: 116, syn. nov.

*Basidiomata* secotioid, semihypogeous to epigeous, up to 35 mm diameter, pulvinate, hemispheric or irregularly globose to subglobose, often with a depressed apex; margin not striate, but at times radially rugose near the stipe, incurved in section and indented around the stipe. *Pileal surface* dry, dull, glabrous to finely pruinose or granulose in the centre, at times with very small scales, rarely subviscid when wet, rosy red, rosy whitish to white towards the margin. *Trama* thin, white, unchanging. Odor pleasant, sometimes aromatic or fruity. Taste mild or very slightly acrid. *Gleba* sublamellate to sublacunose; formed of convoluted lamellae that are joined by cross walls forming elongate to irregular spaces or locules; often exposed near the stipe or at times partially covered by a thin pellicle; yellowish cream to yellow. *Stipe* 5-15 × 3-10 mm, cylindrical; surface dry, dull, rugulose longitudinally, white or rosy at base, cavernous.

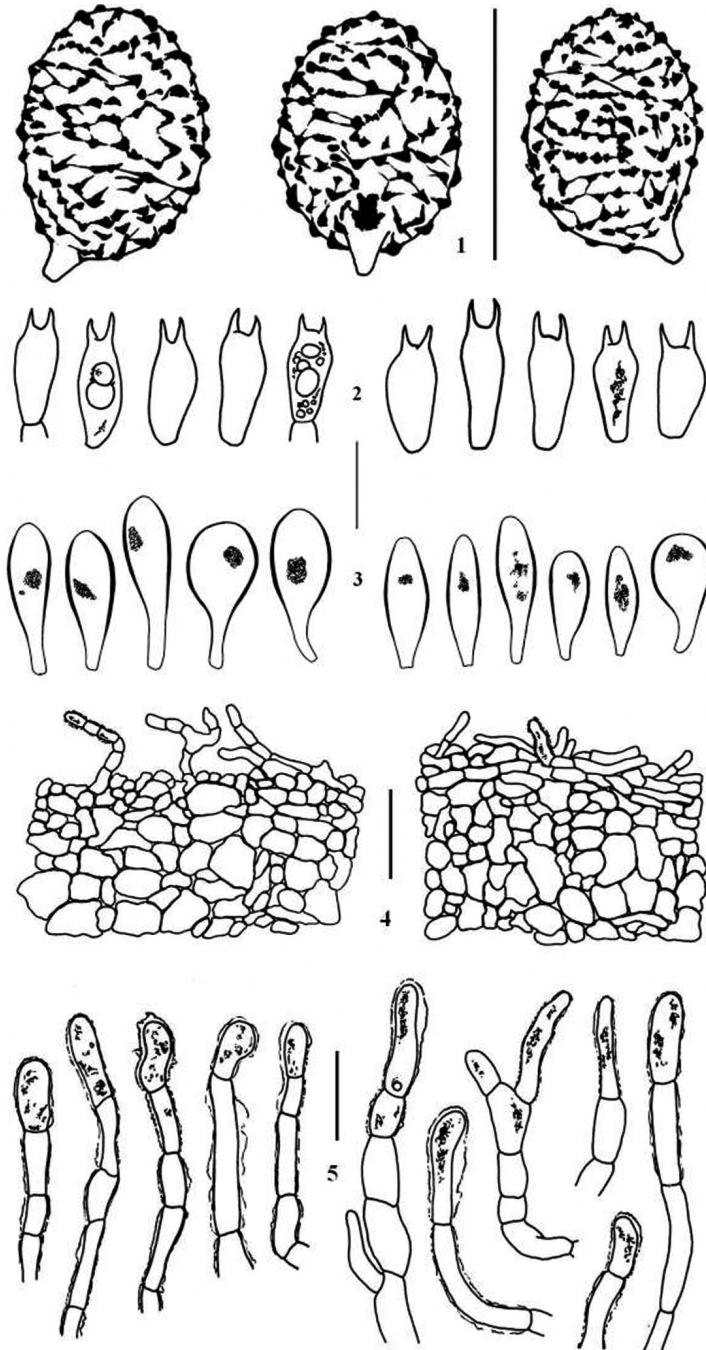


Fig. 1-5. *Cystangium pinetti*. 1. Spores. 2. Basidia. 3. Cystidia. 4. Pileipellis. 5. Terminal hyphae of suprapellis. Drawings left to the scale bar are from holotype, those right to the scale bar are from holotype of *Macowanites mexicanus*. Scale bars: 1, 5 = 10  $\mu$ m. 2, 3 = 20  $\mu$ m. 4 = 50  $\mu$ m.

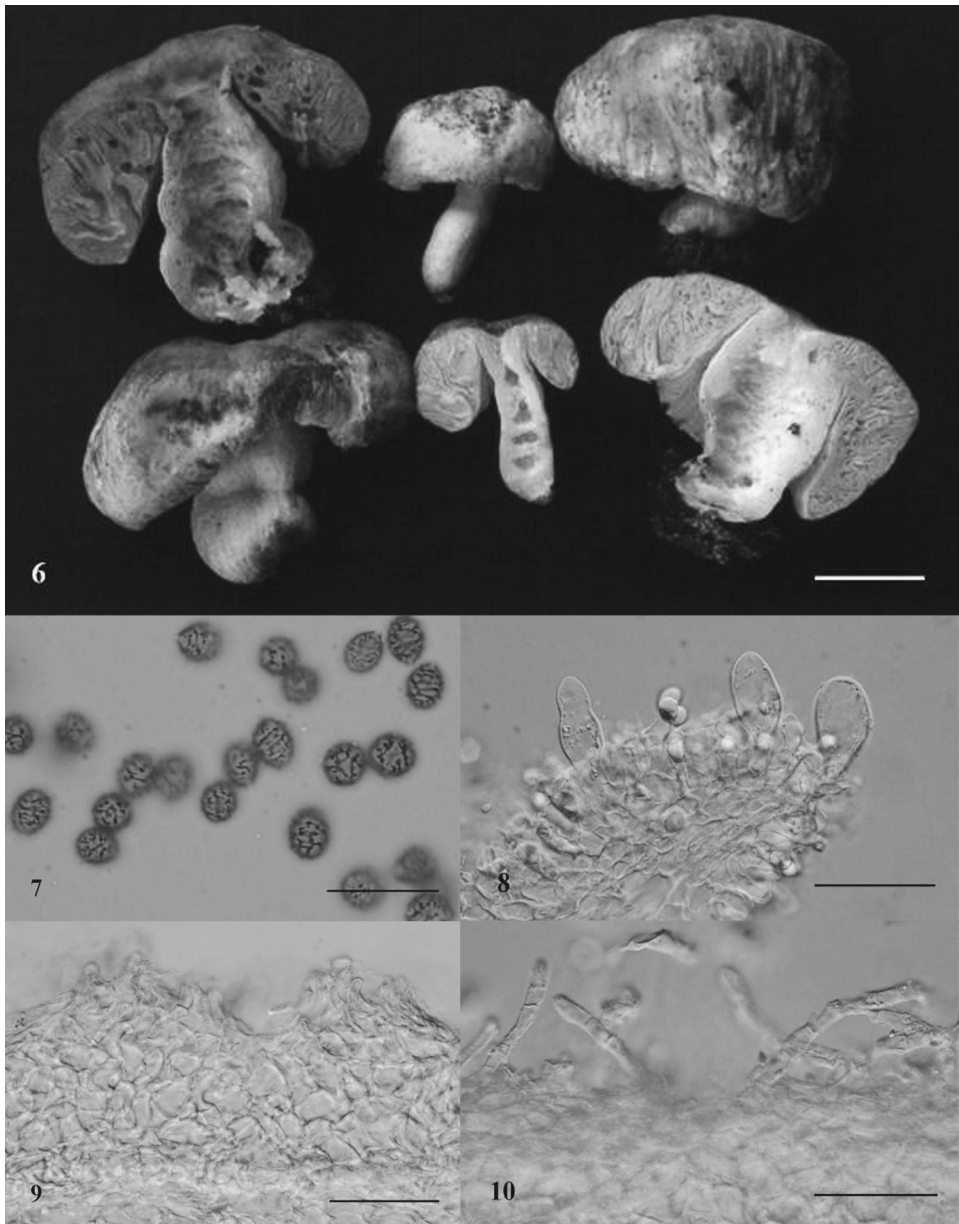


Fig. 6-11. *Cystangium pineti*. 6. Basidiomata (A. Kong 3039). 7. Spores (A. Kong 1746). 8. Hymenial cystidia (A. Kong 2989). 9. Pileipellis (A. Kong 1746). 10. Terminal hyphae of suprapellis (A. Kong 2989). Scale bar: 6 = 1 cm. 7 = 20  $\mu$ m. 8, 9 & 10 = 40  $\mu$ m.

*Basidiospores* 9.0-9.9-10.9-13.1  $\times$  7.1-8.0-8.4-9.5  $\mu$ m including ornamentation (7.5-8.4-9.1-10.7  $\times$  6.3-7.1-7.2-8.3  $\mu$ m; n = 220), ellipsoid (Q = 1.13-1.23-1.29-1.45), heterotropic and asymmetrical; ornamentation amyloid, 0.5-1.0  $\mu$ m high, constituted by verrucae that are often fused, forming small crests or small ridges,

or united by fine lines forming a partial to almost complete reticulum, intermixed with isolated elements; suprahilar plage not well defined. *Basidia* 18-23-25-33 × 8-9-10-12 μm, cylindrical to subventricose or claviform, mainly with two sterigmata. *Cystidia* not abundant but distinctly projecting beyond the basidia, 35-54-70-80 × 11-17-22-28 μm, ventricose to claviform or sphaeropedunculate, thin to slightly thick-walled in the middle portion, empty or with very few amorphous contents, with no reaction in SV. *Subhymenium* a cellular layer, of 1-3 inflated to isodiametrical cells measuring 8-10 μm. *Hymenophoral trama* 42-67 μm wide, of interwoven, hyaline, 3-5 μm wide hyphae; sphaerocytes absent or present in tramal anastomoses; without endomacrocystidia. *Pileipellis* 64-89-141-157 μm thick, two-layered, a hyphoepithelium to a trichoeplithelium, not or slightly gelatinized; suprapellis a thin inconspicuous layer of filamentous hyphae, sometimes combined with inflated cells; hyphal extremities 4.0-5.3-6.7-9.0 μm diam., filamentous, sometimes not forming a distinctive continuous layer, similar to primordial hyphae of *Russula*, septate, slightly thick-walled, at times with hyaline and amorphous incrustated material around terminal segments, without reaction in basic fuchsine or SV, sometimes arranged in compact groups forming small scales or granules on the pileal surface, without macrocystidia; subpellis thick, a pseudo-parenchymatose layer or irregular epithelium, well-developed, composed of strongly inflated or spherical cells, 15-31-38-48 μm thick, thin to slightly thick-walled, part of the cells with a vacuolar, pinkish and diffuse pigment; macrocystidia none. *Stipitipellis* a thin cutis; hyphae 3.4-4.8-6.4(-7.0) μm, hyaline, thin-walled, interwoven, locally inflated and subventricose, 6.5-10.7-15.2(-17.6) μm; terminal hyphae 15-35-58(-64) × 5.1-8.8-13.2(-14.8) μm, cylindrical, subventricose to lageniform; macrocystidia none. *Laticiferous hyphae* and *clamp connections* absent from all tissues.

**Habitat:** Partially buried (semihypogeous) to completely emergent (epigeous), solitary to subgregarious, under different species of *Pinus* (*P. hartwegii*, *P. montezumae*, *P. oocarpa*), between 2800 to 3500 m altitude. **Phenology:** The type specimen of *Cystangium pineti* was collected in October, while that of *Macowanites mexicanus* was found in July. Materials from LMNP have been collected mainly in September and October. **Distribution:** Currently known from Cofre de Perote (type locality), La Malinche, and Popocatepetl Volcanoes. Probably this species grows also on other mountains or volcanoes of the Transmexican Volcanic Belt in Central Mexico, forming ectomycorrhizae with species of the genus *Pinus*.

**Collections examined:** Mexico, Tlaxcala, Huamantla Municipality, LMNP, E slope of La Malinche Volcano, Cañada Grande, 19 September 1990, *A. Kong* 1788; *ibid.*, San Luis Teolocholco Municipality, LMNP, W slope of La Malinche Volcano, 5 October 1998, *A. Kong* 3039; *ibid.*, Trinidad Sánchez Santos Municipality, LMNP, SE slope of La Malinche Volcano, 4-7 Km W of Francisco Javier Mina, 3 September 1998, *A. Kong* 2989; *ibid.*, 14 September 1998, *A. Kong* 2991 (all in TLXM). VERACRUZ: 5 Km S of Las Vigas (as "Los Vigos") along road to Las Lajas, 30 October 1982, *R. Singer* M9514 (F 1052225, holotype of *Cystangium pineti*); area of Cofre de Perote, Las Vigas to Llanillo road, 100 m below Llanillo, 16 July 1981, *G. Guzmán* 19492 (XAL, holotype of *Macowanites mexicanus*).

**Commentary:** *Cystangium pineti* is characterized by the red to rosy red pileal surface, which is often minutely granulose in the centre, combined with a yellowish, sublamellate gleba and stipitate basidiomata. Microscopically the combination of partially reticulate, elliptic spores, two-spored basidia and the presence of hymenial cystidia and encrusted hyphae on the pileus surface distinguish this species. In addition, the association with *Pinus* in coniferous or mixed forests of the Transmexican Volcanic Belt in Central Mexico is a distinctive ecological and biogeographical feature.

## DISCUSSION

Singer (1985) indicated that *C. rodwayi* (Masse) A.H. Sm. is the phenetically closest species to *C. pineti*, due to its spore ornamentation, and development of suprapellis. However, it differs from the Mexican species in the pale brown with grayish rusty red patches on the pileal surface, 4-spored basidia, and in its association with Australian eucalypts. Since the publication of *C. pineti*, eight North American species of sequestrate russuloid taxa (mainly *Martellia* and *Macowanites*) have been described and later transferred within *Cystangium* (Trappe & Castellano, 1986; Cázares & Trappe, 1991; Trappe *et al.* 2002): *C. maculatum* (Singer & A.H. Sm.) Trappe, T. Lebel & Castellano, *C. medlockii* (Trappe & Castellano) Trappe, T. Lebel & Castellano, *C. oregonense* (Zeller) Trappe, T. Lebel & Castellano, *C. variabilisporum* (Singer & A.H. Sm.) Trappe, T. Lebel & Castellano, and *C. vesiculosum* (Singer & A.H. Sm.) Trappe, T. Lebel & Castellano all have a white to pallid pileal surface, astipitate basidiomata, and acystidiate hymenium (except *C. oregonense*, which has moniliform cystidia) (Zeller, 1941; Singer & Smith, 1960; Trappe & Castellano, 1986).

*C. echinosporum* (Zeller & C.W. Dodge) Trappe, T. Lebel & Castellano differs mainly in his salmon-colored basidiomata, small (6-8  $\mu\text{m}$ ) and echinulate spores, and is associated with *Quercus* (Zeller & Dodge, 1919). *C. idahoensis* (Singer & A.H. Sm.) Trappe, T. Lebel & Castellano was described with a white to dead white pileal surface with pale alutaceous tones in some areas, white to pale cinnamon buff gleba, poorly developed columella, astipitate basidiomata, and growing under *Picea* and *Abies* (Singer & Smith, 1960). *C. lymanensis* (Cázares & Trappe) Trappe, T. Lebel & Castellano has pale dull yellow pileal surface with brown stains, more isolated spore ornamentation, acystidiate hymenium, and grows in association with *Abies* (Cázares & Trappe, 1991). Species described from South America (*C. thaxteri* (Singer) Trappe, T. Lebel & Castellano, and *C. depauperatum* Singer & A.H. Sm.) are associated with *Nothofagus*, and have echinulate spores (Singer, 1969, 1985).

Lebel (2003) provided a key to *Cystangium* species from Australia, and *C. pineti* keys out as *C. theodoroui*, from which it differs however by shape and ornamentation of spores, the bisporic basidia as well as by its ectomycorrhizal host association (*Eucalyptus* and *Allocasuarina* for the latter). The only Australian species with two-spored basidia are *C. bisporum* and *C. trappei*, but these have globose or subglobose spores that are orthotropic and ornamented with isolated spines (Lebel, 2003).

Guzmán (1988) pointed out that *Cystangium pineti* differs mainly from *Macowanites mexicanus* by the size of its spores and hymenial macrocystidia. Sizes of spores, basidia, and hymenial cystidia for both type specimens as well as from collections from Tlaxcala are summarized in Table 1. The spore measurements given by both Singer (1985) and Guzmán (1988) included height of ornamentation, and the spores of *M. mexicanus* type are slightly bigger than those of both the type of *C. pineti* type and our own collections from Tlaxcala State. However, the differences between the mean values from all collections do not exceed 0.5  $\mu\text{m}$ . In addition, all collections have mainly bisporic basidia. Also the size of hymenial cystidia is similar for all collections with those of the type of *C. pineti* being only slightly larger.

The type specimen of *Macowanites mexicanus* has a pileipellis with an epithelial structure as in *Cystangium pineti* (Fig. 4). Both type specimens have a poorly differentiated suprapellis of filamentous cells. The pileipellis of *M. mexicanus*

Table 1. Size of spores, basidia, and cystidia in *Cystangium pineti* ( $\mu\text{m}$ ).

	<i>Cystangium pineti</i>		<i>Macowanites mexicanus</i>		Collections from Tlaxcala
	Singer (1985)	Our measurements	Guzmán (1988)	Our measurements	
<i>Spores*</i>					
Length	8.5-10.5	9.0- <u>10.2</u> -11.8	(9-)10-12(-13)	9.0- <u>10.9</u> -13.1	9.0- <u>9.9</u> -11.5
Wide	7.5-8.5	7.5- <u>8.3</u> -9.5	(6-)7-9(-11)	7.1- <u>8.4</u> -9.5	7.1- <u>8.0</u> -9.1
Shape Q	-	1.13- <u>1.23</u> -1.32	-	1.19- <u>1.29</u> -1.44	1.13- <u>1.25</u> -1.34
<i>Basidia</i>					
Length	-	19- <u>25</u> -31	24-30	18- <u>25</u> -33	18- <u>23</u> -28
Wide	-	8- <u>10</u> -12	9-13	8- <u>10</u> -12	8- <u>9</u> -11
<i>Cystidia</i>					
Length	64-80	55- <u>70</u> -82	45-65	65- <u>54</u> -78	44- <u>55</u> -70
Wide	18-19	14- <u>20</u> -27	17-26	11- <u>17</u> -21	16- <u>22</u> -28

was described as not well defined and composed by filamentous elements and dispersed dermatocystidia (Guzmán, 1988). However, the latter description was only based on the suprapellis elements and ignored the subjacent elements, in particular the presence of a pseudoparenchymatic subpellis, which explains its original assignment to *Macowanites*. Based on our own examination of the types, we therefore conclude that *M. mexicanus* and *C. pineti* are one and the same species and the name *C. pineti* should be adopted for both.

The pileus suprapellis of *Cystangium pineti* presents undeniable similarities to that of *Russula* subgenus *Incrustatula* Romagnesi (1987; section *Lilaceae sensu* Bon, 1988). The filamentous cells of the suprapellis are morphologically very similar to the primordial hyphae described for *Russula* subgenus *Incrustatula*: they are long, septate, cylindrical, and often encrusted with irregular hyaline to waxy-like material around the terminal segments (Fig. 5). However, the encrusted material or the hyphae do not stain purple red to deeply magenta with basic fuchsin (not acid resistant applying the differential method of Melzer). Also the SV reaction results in reddish to bright pink macrochemical reaction on the dry stipe, similar to that recorded for species of subsections *Roseinae* or *Amethystinae*. These microscopical and chemical features have never been recorded in other *Cystangium* species and suggest a close relationship with the above-mentioned *Russula* (sub)sections. In addition, some species of *Russula* (sub)sections *Roseinae* or *Amethystinae* are also similar in having a pseudoparenchymatic subpellis, without pileomacrocytidia, and in some cases their hymenial macrocytidia also do not react with SV.

Recent molecular evidence indicates that the sequestrate genera of *Russulaceae* belong to clades that are clearly polyphyletic and comprise also agaricoid taxa (Miller *et al.*, 2001; Eberhardt & Verbeken, 2004; Lebel & Tonkin, 2007). Molecular studies may therefore reveal a close relationship between *Cystangium pineti* and members of the above-mentioned groups in *Russula*.

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\*Measurements included ornamentation.