



# *Kavinia altoandina* (Basidiomycota, Gomphales), a new species from the Andean Mountains of northern Chile

*Kavinia altoandina* (Basidiomycota, Gomphales) una nueva especie de ambientes altoandinos de Chile

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# ABSTRACT

Kavinia altoandina is a new species from the Andean Mountains in northern Chile. It is characterized by a hydnoid hymenophore that is white to cream when young but olive green when mature, as well as by the cylindrical to fusiform, verrucose basidiospores, 7.5 11.8  $\times$  3.3 4.5  $\mu$ m. In addition, phylogenetic relationships inferred from internal transcribed spacer (ITS) sequences support K. altoandina as a new species related to K. chacoserrana from Argentina.

Keywords — Chilean mycobiota; corticioid fungi; hydnoid fungi; phylogeny; taxonomy.

# RESUMEN

Se describe *Kavinia altoandina* como una nueva especie con base en datos morfológicos y evidencia molecular. La especie fue recolectada en ambientes andinos del norte de Chile, y se caracteriza morfológicamente por su himenóforo hidnoide, blanquecino cuando joven a verdoso cuando maduro. Microscópicamente se caracteriza por sus basidiosporas cilíndricas a fusiformes, 7.5 11.8 × 3.3 4.5  $\mu$ m. Además de los datos morfológicos, las relaciones filogenéticas inferidas de las secuencias del

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espaciador transcrito interno (ITS) respaldan a *K. altoandina* como una nueva especie relacionada con *K. chacoserrana*.

Keywords — Micobiota chilena; hongos corticioides; hongos hidnoides; filogenia; taxonomía.

# INTRODUCTION

*Kavinia* Pilát is a corticioid genus characterized by resupinate, hydnoid basidiomes with a loose subiculum bearing hymenial aculei. Microscopically by a monomitic hyphal system with clamp connections, fusiform, cylindrical or subcylindrical basidiospores ornamented with cyanophilous warts. (Eriksson & Ryvarden, 1976; Boidin & Gilles, 2000; Bernicchia & Gorjón, 2010; Robledo & Urcelay, 2017). Phylogenetically, *Kavinia* belongs in the Lentariaceae in the Gomphales (Hosaka *et al.*, 2006; Giachini *et al.*, 2010). Within in the Lentariaceae, *Kavinia* and *Hydnocristella* (R.H. Petersen) are the only genera that develop resupinate basidiomes, but they are phylogenetically and morphologically distinct producing ornamented and smooth basidiospores, respectively (Larsson, 2007).

Five Kavinia species are currently recognized: K. alboviridis (Morgan) Gilb & Budington, K. chacoserrana Robledo & Urcelay from Argentina, K. globispora Natarajan & Koland. from India, K. salmonea Boidin & Gilles from Reunion, and K. vivantii Boidin & Gilles from Guadeloupe. Of these species, only K. alboviridis is widely distributed with numerous records from both hemispheres (Boidin & Gilles, 2000; Bernicchia & Gorjón, 2010; Doðan, 2009; Kout & Hajšmanová, 2015), but mainly in temperate regions (Eriksson & Ryvarden, 1976). In contrast, the other species are only known from their type locations (Natarajan & Kolandavelu, 1985; Boidin & Gilles, 2000; Robledo & Urcelay, 2017). New genetic evidence suggests that K. alboviridis encompasses multiple phylogenetic species (Kout & Hajšmanová, 2015; Robledo & Urcelay, 2017).

The mycobiota from northern Chile is poorly known, especially from the highaltitude environments in the Andes Mountain range. Only a few studies have reported on the fungi of this region (e.g., Sandoval-Leiva *et al.*, 2017; Troncoso *et al.*, 2020). During studies on the mycobiota in the Andes Mountain range of northern Chile, we collected specimens that resembled *K. alboviridis*. The specimens were found in shrublands that are exposed to extreme climatic conditions, such as high temperature and solar radiation during the summer and low temperatures in winter. Subsequent morphological and molecular analyses revealed that the specimens represented an undescribed species of *Kavinia*. We contribute to the knowledge of fungi from this unique environment of northern Chile by describing *Kavinia altoandina*, a new species based on morphology and phylogenic evidence of the ITS rDNA.

# MATERIALS AND METHODS

# Collections

Samples were collected and processed according to Rossman *et al.* (1998). Macroscopic descriptions are based on fresh material according to Lodge *et al.* (2004). Microscopic features are described from material mounted in KOH (5%), Congo Red (1%), Melzer's reagent and Cotton Blue. Basidiospore statistics include  $x_m$ , the arithmetic mean of the spore length by spore width (± SD) for n basidiospores spores measured; Q, the ratio of spore length to spore width, expressed as a range for all basidiospores measured; Q<sub>m</sub>, the mean of all Q values (± SD). Microscopic measurements were presented as format of (min–) 5th–95th percentile (–max). Voucher specimens have been deposited in the Herbarium of the Museo Nacional de Historia Natural de Santiago, Chile (SGO). Herbarium acronyms follow Thiers (2022).

## DNA extraction and sequencing

DNA was extracted from specimens using the Extract N Amp Plant kit (Sigma-Aldrich, St. Louis, Missouri) and the ITS region was amplified and sequenced using primers ITS1F and ITS4 following White *et al.* (1990). For PCR of ITS and 28S, we used Taq Polymerase with Standard Taq Buffer (New England Biolabs, Ipswich, Massachusetts) following the manufacturer's protocols.

# **Phylogenetic analyses**

ITS sequences generated in the present work were combined with those retrieved from GenBank (NCBI) to construct a dataset of 55 taxa. Scientific names and Gen-Bank accession numbers of sequences are listed in Table 1. *Clavaria delphus* Donk was selected as outgroup (Hosaka *et al.*, 2006; Giachini *et al.*, 2010; Robledo & Urcelay, 2017).

Phylogenetic analyses were performed following Robledo *et al.* (2021). ITS sequences were aligned using MAFFT 7 (Katoh & Standley, 2013) using the G-INS-i alignment method. Alignments were manually inspected and adjusted using MEGA 6 (Tamura *et al.*, 2013). ModelFinder (Kalyaanamoorthy *et al.*, 2017) as implemented in the IQ-Tree software (Nguyen *et al.*, 2015) was used to estimate the best-fit partitioning strategy and the best-fit model of nucleotide evolution for the dataset using three data blocks (ITS1; 5.8S; ITS2). Models were restricted for those implemented in MrBayes 3.2 (Ronquist *et al.*, 2012). Bayesian inference (BI) and maximum likelihood (ML) phylogenetic analyses were applied to the dataset using the partition scheme and evolutionary models defined by ModelFinder. BI was performed following Robledo *et al.* (2020, 2021) in the CIPRES science gateway (Miller *et al.*, 2010; http://www.phylo.org/). Maximum likelihood searches were conducted with IQ-Tree. The analysis initially involved 100 ML searches, each one starting from one randomized stepwise addition parsimony tree. Branch supports were calculated using the UFBoot (ultrafast bootstrap approximation) (Hoang *et al.*, 2018) implemented

**Table 1 (part 1 of 2).** List of taxa, specimens and sequences used in the phylogenetic analysis. New sequences generated in this study are in boldface. T = Type specimen.

 

 Tabla 1 (parte 1 de 2). Lista de taxones, especímenes y secuencias usadas en los análisis filogenéticos. Las nuevas secuencias generadas en este estudio están en negrita. T = espécimen tipo.

Clavariadelphus occidentalis       EU846242         OSC 114281, USA       EU8669308         H21536, Tunisia       KU973835         Clavariadelphus pistillaris       Itelefeessot         3894, Canada, Quebec       KM215935         Clavariadelphus pistillaris       Itelefeessot         SM1278, Canada, Quebec       KM2159395         Clavariadelphus truncatus       Itelefeessot         SM1278, Canada, British Columbia       MC836048         UBC:F21557, Canada, British Columbia       MZ836049         Lentaria bambusina       MH8027302, Liuyang, Hunan, China       KU324496         MHHNU 6794, Sangzhi, Hunan, China       KU870448       Itentaria byssisseda         TENN61159, USA, TN       FJ596785       RA5454, USA: Tennessee       MT196983         Lentaria gassypina       UNAM:FCME27625, Mexico: Veracruz,       MK253199       UNAM:FCME27626, Mexico: Veracruz,       MK253200         Lentaria, aff. micheneri       MHNU 7829, China: Baihaba, Xinjiang       KU324498       MMAU226892, China: Inner Mongolia       AU292289         Lentaria aurializati       MHNU 7829, China: Inner Mongolia       KU870449       MA54049         MAFungi 48032, Spain       AU292290       MMA5706       MM871706         MushroomObserver.org/355909, USA, California       MW870450       Itenta	Taxón Voucher reference – Origin	Genbank / Putoff Accesion Nº – ITS
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MA-Fungi 48091, SPAIN AJ292291	MA-Fungi 48091, SPAIN	AJ292291
Hvdnocristella latihvpha	Hvdnocristella latihvpha	
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He 20120914-4 China, Jiuzhaigou Nature Reserve KM489522	He 20120914-4 China, Jiuzhaigou Nature Reserve	KM489522
Kavinia aff. alboviridis	Kavinia aff. alboviridis	
UC2022816, USA, AK KP814530	UC2022816, USA, AK	KP814530

### Table 1 (part 2 of 2).

#### Tabla 1 (parte 2 de 2).

Taxón Voucher reference – Origin	Genbank / Putoff Accesion Nº – ITS
Kavinia alboviridis	
KM82737, United Kingdom: England	GQ981505
KM141510, United Kingdom: England	GQ981506
K(M)257290, United Kingdom: Wales, Anglesey	MZ159756
Kavinia chacoserrana	
Robledo 2516, Argentina, Córdoba (T)	NR164256
Kavinia altoandina	
PSL2302, Chile (T)	OP022196
PSL3014, Chile	OP022197
Kavinia sp	
G4620, Estonia	UDB0288438
G4761, Estonia	UDB0445985
G4278, Estonia	UDB0512813
G4646, Estonia	UDB0428073
G2558, Estonia	UDB0437262
G4177, Estonia	UDB0484646
G4705, Estonia	UDB0657677
G4762, Estonia	UDB0455445
G4459, Estonia	UDB0238831
Ramaricium polyporoideum	
TENN:056036, Argentina: Tierra del Fuego	KY352646
TENN:065654, USA: North Carolina	MF992160

in IQ-TREE with 1000 replications. A node was considered strongly supported with BPP  $\ge 0.95$  or BS  $\ge 95\%$  (Hyde *et al.*, 2013; Minh *et al.*, 2020). Hereafter, support values are presented as BPP/BS in the text.

## RESULTS

# **Phylogenetic analyses**

The final dataset included 55 terminals and 688 characters, of which 299 were parsimony informative and 369 were constant. The partitions and evolutionary models selected were K2P+I (5.8S) and SYM+I+G (ITS1 and ITS2). Bayesian and ML analyses resulted in similar topologies; the Bayesian consensus tree is presented in Figure 1. The final topology resolved *Kavinia* specimens from high altitude environments in northern Chile as a well-supported, independent lineage within the genus *Kavinia*. There was strong bootstrap support for a sister relationship between the Chilean *Kavinia* specimens and the Argentinian species *K. chacoserrana* (1/99, Figure 1).





**Fig. 1.** Árbol de consenso estricto de Inferencia Bayesiana (BI) con base en secuencias ITS. Los valores de soporte de las ramas se muestran como BPP/BS, probabilidad posterior bayesiana superior a 0,7 y valores de Bootstrap superiores al 70 %.  $\blacktriangleleft$  T = espécimen tipo.

# Taxonomy

# Kavinia altoandina Sandoval-Leiva & Calle sp. nov. Figs. 2-3 Mycobank: MB844867

**Diagnosis.**— Hymenophore hydnoid, dark olive-green; Subiculum whitish to cream, arachnoid to cottony; basidospores verrucose, fusiform to cylindrical, 7.5 11.8  $\times$  3.3 4.5  $\mu$ m; ocurring on dead and partially buried wood of *Adesmia hystrix*, restricted to high Andean environments of the Atacama region in Chile.



**Fig. 2.** *Kavinia altoandina*. A) Location of the holotype of *K. altoandina* in a scrubland of *Adesmia hystrix* in the Andes Mountains of Chile. B-D) Macromorpholgy of *Kavinia altoandina* (P. Sandoval-Leiva 2302, holotype). B) Developing aculei arising from arachnoid subiculum. C & D) General view of hymenophore *in situ*. D) Clustered aculei covered by mature basidiospores. Scale bars B-D = 5 mm.

**Fig. 2.** *Kavinia altoandina*. A) Holotipo de *K. altoandina* en un matorral de *Adesmia hystrix* en la Cordillera de los Andes de Chile. B-D) Macromorfología de *Kavinia altoandina* (P. Sandoval-Leiva 2302, holotipo). B) Dientes en desarrollo surgiendo del subículo aracnoideo. C y D) Vista general del himenóforo *in situ*. D) Agujas agrupadas cubiertas por basidiosporas maduras. Escala B-D = 5 mm.



**Fig. 3.** Microscopic features of *Kavinia altoandina*. A) Loosely interwoven generative hyphae with ampuliform clamps from the subiculum. B-C) Crystalline incrustations in subiculum. D) Close-up of hymenium. E-F) Detail of basidiospores and basidia. Scale bars =  $10 \ \mu m$ .

**Fig. 3.** Características microscópicas de *Kavinia altoandina*. A) Hifas generativas del subículo laxamente entrelazadas con fíbulas ampuliformes. B-C) Incrustaciones cristalinas en el subículo. D) Detalle del himenio. E-F) Basidiosporas y basidios en detalle. Escala = 10  $\mu$ m.

**Typification.**— CHILE. Región de Atacama: Province Huasco, Municipality Alto del Carmen, hillsides from quebrada Los Barriales, 29°15'51.8" S, 70°5'2.9" W, 3.633 m asl, on fallen and buried dead wood of *Adesmia hystrix* in a scrubland of *A. hystrix*, 16-XI-2017, *P. Sandoval-Leiva 2302* (holotype, SGO). GenBank OP022196 (ITS).

**Etymology.**— The epithet "altoandino" is a phytogeographical term that refers to high altitude environment in the Andes Mountains and its vegetation.

**Basidiomata** annual, resupinate, loosely attached, up to  $23 \times 7$  cm, arising from several points at the substrate and later often confluent. **Hymenophore** hydnoid, aculei narrowly conical up to 3 mm long and up 0.5 mm diam at the base, white to cream when immature (Fig 2C), dark olive green at maturity (Fig 2B, D), smooth, subceraceous, scattered and clustered; apex sterile, subulate, paler. **Subiculum** whitish to cream, loose, arachnoid to cottony, becoming more compact with age, thin, sterile. **Margin** distinct, byssoid or fibrillose, white to cream, sterile, with white, sparsely branched rhizomorphs.

**Hyphal system** monomitic. Generative hyphae clamped, clamps often ampuliform, hyaline and thin- to slightly thick-walled, commonly up to 5  $\mu$ m diam, occasionally swollen up to 9  $\mu$ m. Hyphae regular and parallelly arranged in the trama

of aculei, loosely interwoven in the subiculum. Crystalline incrustations abundant in subiculum and rhizomorphs, scarce in aculei.

**Cystidia** absent. **Basidia**  $(23.7-)29.5 - 39.2(-42.3) \times (5.3-)6-8.6(-8.8) \mu m$  ( $x = 33.9 \times 7.2 \mu m$ ), clavate, with a basal clamp, hyaline, commonly with guttules, 4-sterigmata up to 7  $\mu$ m long. **Basidiospores** fusiform to cylindrical in side view or ellipsoid in dorsi-ventral view, with a distinctly supra-apicular concavity and a rounded to tapering apex, occasionally blunt or truncate, (6.9 )7.5 11.8( 13.4) × (3.1 )3.3 4.5( 4.9) [ $x_m = 8.97 \pm 1.20 \times 3.82 \pm 0.34 \mu m$ , Q =(1.83 )2.3 2.4 ( 3.82), Q<sub>m</sub> = 2.35 \pm 0.30, n = 200], slightly thick-walled, pale yellow to olivaceous yellow, verrucose, with cyanophilous ornamentations, IKI-, occasionally grouped in tetrads.

**Distribution**.— Known from the Andean Mountains in the Atacama region of Chile.

**Specimen studied.**— CHILE. Región de Atacama: Province Huasco, Municipality Alto del Carmen, hillsides from quebrada Los Barriales, 29°15'10.4" S, 70°5'42.6" W, 3.534 m asl, on fallen and buried dead wood of *Adesmia hystrix* in a scrubland of *A. hystrix*, 17-XI-2017, *P. Sandoval-Leiva 3014* (SGO). GenBank OP022197 (ITS).

Additional specimen studied.— Kavinia chacoserrana: ARGENTINA, Córdoba, Dpto. San Alberto, Los Hornillos, on dead fallen branch of Lithraea molleoides, 31°54'0.8"S, 64°58"0.6"W, 1280 m asl, 28-IV-2012, Robledo 2516 (holotype CORD). GenBank MF377531 (ITS).

#### DISCUSSION

Kavinia altoandina is most similar to K. alboviridis that also has an olivaceous green, hydnoid hymenophore at maturity but with slightly shorter basidiospores. Its closest phylogenetic relative, however, is K. chacoserrana that differs by its white to pale hymenophore. In addition, current genetic evidence suggests that K. alboviridis is a species complex involving several taxa (Kout & Hajšmanová, 2015; Robledo & Urcelay, 2017). Further molecular and morphological studies are needed to resolve the taxonomic status of this species and its synonyms. The broad distribution of K. alboviridis records from both hemispheres is unusual and may represent several different species with restricted distributions.

Although morphologically, K. altoandina is similar to K. alboviridis the former ecological niche is unique amongst Kavinia species because it is restricted to high altitude Andean environments of the Atacama region in Chile. Kavinia altoandina has adapted to the extreme temperature variation and high solar radiation present in high elevation areas over 3000 m asl by growing on dead, partially buried wood of Adesmia hystrix, thus, obtaining some protection from the inclement climate.

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