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New record of *Agaricus subrufescens* (Agaricales, Basidiomycota) for Ecuador

Nuevo registro de *Agaricus subrufescens* (Agaricales, Basidiomycota) para Ecuador

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ABSTRACT

Agaricus subrufescens (Agaricales, Basidiomycota), an edible species with attributed medicinal properties, has been reported from several countries of the world. This study presents the first record for Ecuador, based on morphological and molecular (ITS-5.8S of DNArn) characterization for one wild specimen collected outside the Puyango Petrified Forest (PPF). This information marks the baseline for future research projects in areas such as the Puyango Petrified Forest, declared National Heritage of Ecuador, to valorise neotropical fungi with commercial potential.

Keywords: DNArn; El Oro; ITS-5.8S; Loja; morphology.

RESUMEN

Agaricus subrufescens (Agaricales, Basidiomycota), una especie comestible a la que se atribuyen propiedades medicinales, ha sido reportada en varios países del mundo. Este estudio presenta el primer registro para Ecuador, basado en caracterización morfológica y molecular (ITS-5.8S del DNArn) de un espécimen silvestre colectado fuera del Bosque Petrificado Puyan-

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go (BPP). Esta información marca la línea base para futuros proyectos de investigación en áreas como el Bosque Petrificado Puyango, declarado Patrimonio Nacional del Ecuador, para valorizar hongos neotropicales con potencial comercial.

Palabras clave: DNArn; El Oro; ITS-5.8S; Loja; morfología.

INTRODUCTION

Agaricus subrufescens Peck (Agaricales, Basidiomycota) has been described in several countries of different continents, e.g. North and South America; Asia; Europe; and Oceania, and then considered cosmopolitan (Wasser *et al.*, 2002; Kerrigan, 2005; Zhao *et al.*, 2011; Thongklang *et al.*, 2016; Zied *et al.*, 2021). It is a saprotrophic specie ecologically important for decomposition of organic matter, with reports of high nutritional and medicinal characteristics and commercial importance (Hetland *et al.*, 2011).

Agaricus subrufescens belongs to *Agaricus* section *Arvenses* (Cao *et al.*, 2021), with notable variability in its macromorphology (robust or thin basidiomata) or very variable color on the surface of the pileus, presenting shades ranging from white to golden brown or violet brown, due to the genotype as influences of the climate and ecosystems where it develops (Kerrigan, 2005). Sometimes this species is difficult to distinguish morphologically from other species like *A. macrochlamys* (Medel-Ortiz *et al.*, 2022). Based on molecular information, *A. subrufescens* has been described from USA, and considered conspecific with some *Agaricus* species described from América, Europa and Africa: *A. brasiliensis* Fr. from Brazil; *A. blazei* Murrill from U.S.A; *A. fiardii* Pegler from Martinique; *A. rufotegulis* Nauta from Netherlands; *A. bambusae* Beeli from Belgique (Wasser *et al.*, 2002; Kerrigan, 2005; Thongklang *et al.*, 2016; Ehrich & Hutter, 2020; Zied *et al.*, 2021; Medel-Ortiz *et al.*, 2022).

In South America, *A. subrufescens* has been reported from Brazil (Kerrigan, 2005), and mentioned in commercial production as *A. blazei* in Argentina (González *et al.*, 2011) and in Ecuador Hidalgo & López (2019) report biological activity of a commercial strain *A. blazei*.

So far, no records of the species has been done in fungal diversity works for Ecuador, considered a megadiverse country [United Nations Environment Programme, 2014], (Cruz *et al.*, 2016; Benítez *et al.*, 2021; Gates *et al.*, 2021; Toapanta-Alban *et al.*, 2021). In addition, there are no data on the traditional use of this species by indigenous communities (Andrade *et al.*, 2012; Gamboa-Trujillo *et al.*, 2019). *Agaricus subrufescens* is commercially cultivated in several parts of the world due its nutraceutical importance (Mata & Savoie, 2012; Sánchez & Mata, 2012; Wisitrassameewong *et al.*, 2012; Thongklang *et al.*, 2016; Velázquez-Narváez *et al.*, 2018; Zied *et al.*, 2021).

The Petrified Forest of Puyango (PPF or Puyango Petrified Forest) in Ecuador, a protected ecosystem area, is recognized as National Heritage of Ecuador (Morante-Carballo *et al.*, 2020). So far, no fungal species have been formally recorded in this area using a molecular approach (Læssøe & Petersen, 2008; Ordoñez, 2018; Batallas-Molina *et al.*, 2021; Gates *et al.*, 2021). However, only a preliminary checklist for PPF showed an interesting fungal diversity (Cruz & Masache, 2023).

In this work the species *A. subrufescens* is present as new record based in morphological and molecular data (ITS-5.8S DNArn) for Ecuador.

MATERIALS AND METHODS

Material and studied area.— The specimen of *Agaricus* sp. was found in the outer limit of the PPF, and it was deposited in the mycoteca of the HUTPL Herbarium with the reference number HUTPL(F)2047. The PPF is located in southern region of Ecuador (Fig. 1).

Morphological analyses.— Macroscopic analyses were based on the observation (fresh sample) of the shape, texture and color of the basidiome. Color codes are based on the [https online server https://encycolorpedia.es/a49074](https://encycolorpedia.es/a49074) (last viewed 28 March 2023). Macrochemical reaction on the pileus surface was tested with Potassium hydroxide KOH 10%. For microscopical analyses hand cuts of basidiome were made through a Stemi Carl Zeiss stereomicroscope, and the preparations were stained with 1% Phloxine or 1%

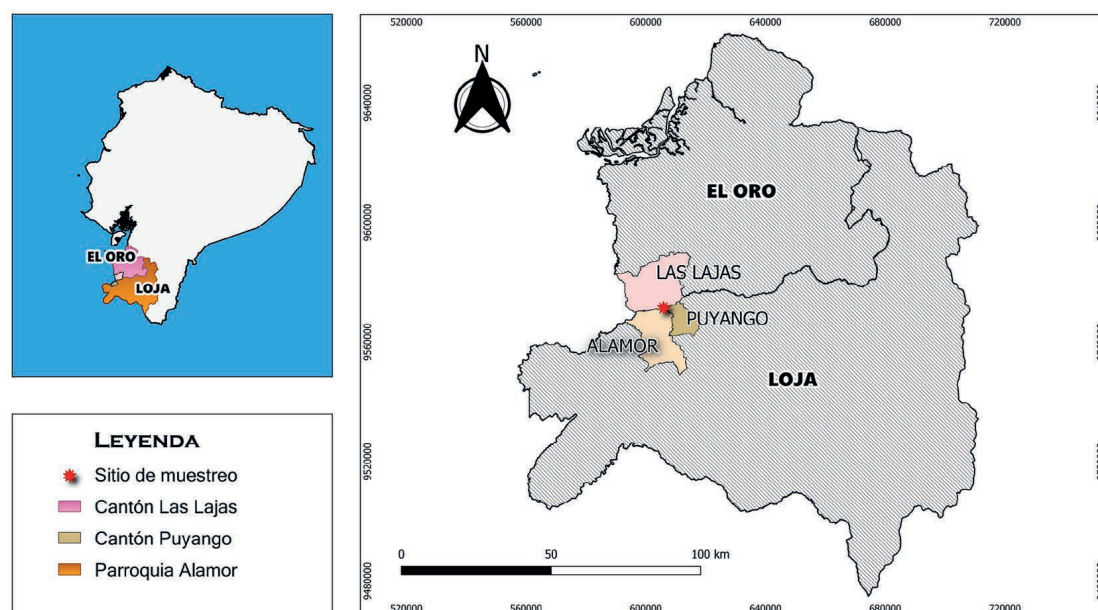


Fig. 1. Geographical Location of the Puyango Petrified Forest.

Fig. 1. Ubicación Geográfica del Bosque Petrificado de Puyango.

Congo red and Melzer's reagent. The samples were examined at 1000-fold (100X) magnification using an Olympus BX51 microscope.

The length and width of microstructures were taken from at least 30 measurements. The range of measurements includes averages ($\bar{x}$) of the most constant values and extreme values in parentheses. The Q-value of basidiospores (average spore length/average spore width) was recorded and basidiospore shape was classified according to Largent *et al.* (1973).

DNA extraction and molecular analysis.— DNA was extracted from the *Agaricus* sp., basidiome under the recommended protocol in the Phire Plant Direct Master Mix PCR Kit (Thermo Scientific™), followed by polymerase chain reaction (PCR) amplification using the unmodified primers ITS1 and NL4 (White *et al.*, 1990). The final reaction volume was 20 μ l including 1 μ l target DNA for the reaction. PCR conditions were as follows: initial denaturation (98 °C, 5 min); followed by 40 cycles with denaturation (98 °C, 10 s); annealing (55 °C, 10 s); extension (72 °C, 30 s); and final extension (72 °C, 5 min). The PCR product was purified with Genomic Plant DNA Purification Kit (Invitrogen™ PureLink™). Sequencing (SANGER) was carried out at Macrogen INC (Seoul-Korea) using the same PCR primer pair.

The sequences were assembled and edited in Codon Code Aligner 5.1.4 (CodonCode Corporation, Centerville, MA, USA). All selected sequences (from basidiomata or strains) mainly correspond to the most similar (about 3%) and sequences named at species level with difference no more than 10% to sequences at the GenBank database (<https://www.ncbi.nlm.nih.gov>) as well as the UNITE database (<https://unite.ut.ee>) (Table 1).

Alignment of the obtained sequence and reference sequences was carried out in the MAFFT 7 program (Kato *et al.*, 2019) under the GINSI algorithm. Phylogenetically, Neighbour joining (NJ) analysis, Kimura 2-parameter (K2P) model with 1000 Bootstrap replicates, and Maximum Likelihood (ML), General Time Reversible (GTR) model with 1000 Bootstrap replicates, constructed in the MEGA X program (Stecher *et al.*, 2020), were performed. The phylogentic tree was outgroup rooted with sequences from *A. sodalis* and *A. megalosporus* belong to section Minores, and the values above the nodes correspond to ML and NJ respectively. The sequence of this study (GenBank accession number ON799184) is available in the National Center for Biotechnology Information (NCBI) database.

Table 1. Sequence information used in this study. The DNA region for all sequences used in the alignment was ITS-5.8S. T= Type specimen.

Tabla 1. Información de secuencia utilizada en este estudio. La región de ADN para todas las secuencias utilizadas en el alineamiento fue ITS-5.8S. T= Especimen tipo.

Taxon	Voucher/Strain	Origin	GenBank
<i>Agaricus abruptibulbus</i>	HKAS 81074	China	KJ755648
<i>Agaricus arvensis</i>	voucher MA Fungi 80999	Spain	KF114474
<i>Agaricus augustus</i>	strain WC908		AY484671
<i>Agaricus augustus</i>	voucher 110116MFBPL0411		MW488346
<i>Agaricus blazei</i>	WC837	Brazil	AY484697
<i>Agaricus blazei</i>	basidiome	North Korea	AF161013
<i>Agaricus blazei</i>	GKVK 18	India	MF403088
<i>Agaricus campestris</i>	NVE 471	Colombia	KF937296
<i>Agaricus crocodilinus</i>	Mushroom Observer #255130 Haplotype 2	USA	MH236110
<i>Agaricus fiardii</i>	F2285 LIP Martinique	France	JF797201
<i>Agaricus flocculosipes</i>	MFLU20140224	Thailand	KP705076
<i>Agaricus gemellatus</i>	HBAU15501		MW862375
<i>Agaricus guizhouensis</i> (T)	voucher HKAS 81081	China	NR_151749
<i>Agaricus julius</i>	strain ZRL20162037	China	MK617912
<i>Agaricus longistipes</i> (T)	voucher HKAS 81066	China	NR_151748
<i>Agaricus megalocarpus</i> (T)	voucher HKAS 71717	China	NR_151747
<i>Agaricus megalosporus</i> (T)	voucher MFLU 100774		NR_119951
<i>Agaricus rufotegulis</i>	strain L 0341732	United Kingdom	AY818649
<i>Agaricus sodalis</i> (T)	strain LD2012159 voucher MFLU 12 0987	Thailand	KP715161
<i>Agaricus</i> sp.	voucher NTF67	Thailand	JF514529
<i>Agaricus subrufescens</i>	strain JSR3	China	MT525362
<i>Agaricus subrufescens</i>	strain WC837 S04 1	Brazil	KJ541796
<i>Agaricus subrufescens</i>	voucher J Erlich BOLETUS	Germany	MT210899
<i>Agaricus subrufescens</i>	strain 610723MF0089	China	KY950519
<i>Agaricus subrufescens</i>	Basidiome HUTPL(F)2047	Ecuador	ON799184
<i>Agaricus subrufescens</i>	strain ABL 16 01	Brazil	MW200292
<i>Agaricus subrufescens</i>	isolate 13cljw3	China	KJ162111
<i>Agaricus subrufescens</i>	isolate 13cljw4	China	KJ162112

RESULTS

Molecular phylogenetic inference

Phylogenetically, 28 sequences were analyzed including our sequence from *Agaricus* sp. HUTPL(F)2047. The sequence of our specimen was clustered within *A. subrufescens* clade (Bootstrap value 99%) into the *Arvenses* section (Fig. 2). The *A. subrufescens* clade include sequences obtained from commercial and wild strains from several countries: *A. rufotegulis* culture from United Kingdom; *A. blazei* commercial and wild strains from Brazil BR; *A. subrufescens* wild specimen from Germany GER; commercial strains from China, North Korea; and a sequence obtained from a basidiome identified as *A. fiardii* voucher F2285 LIP Martinique France.

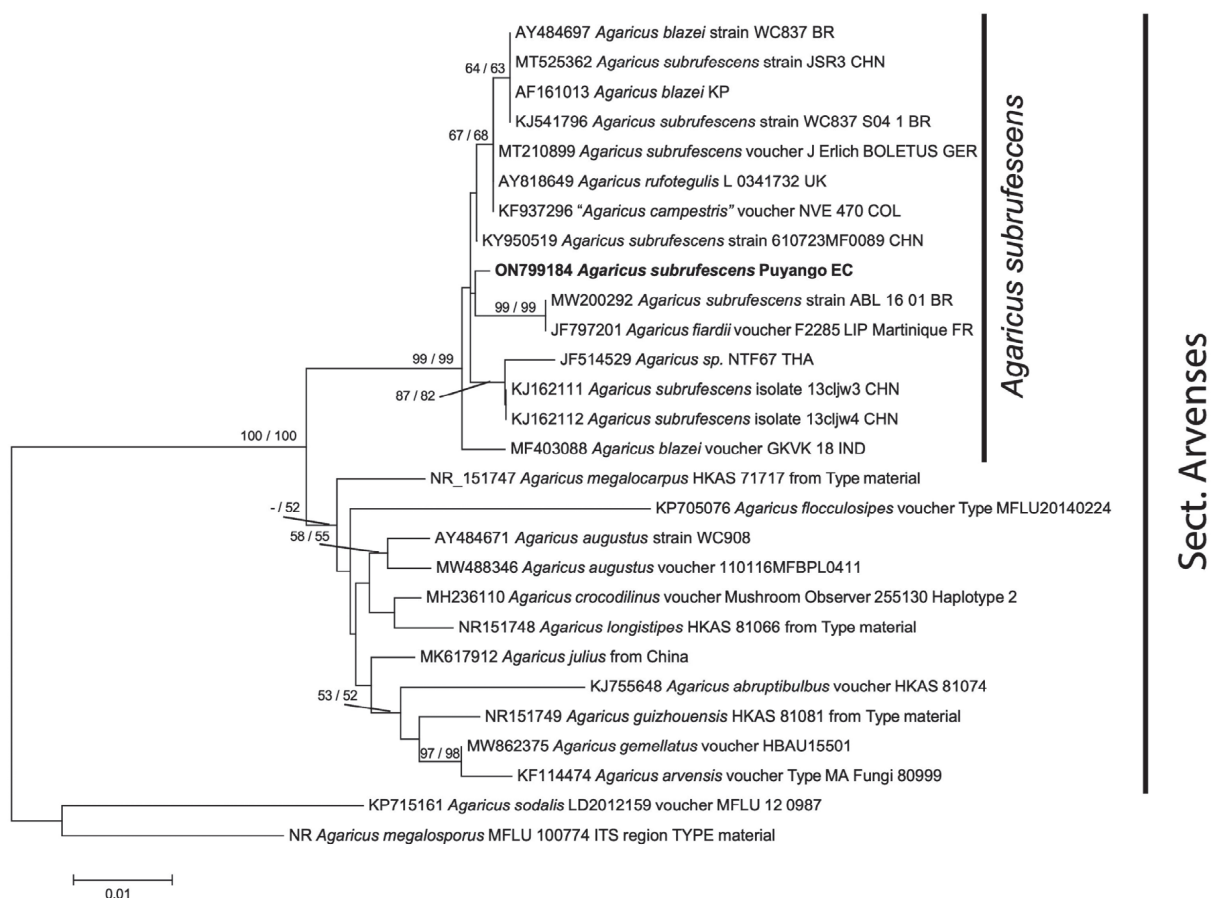


Fig. 2. Phylogenetic tree to the ITS-5.8S region indicating the positioning of the *Agaricus* sp. (bold) within the *Agaricus subrufescens*. Bootstrap values on nodes correspond to Maximum likelihood and Neighbour joining respectively. Midpoint rooting. Bar = number of replacements by position. BR = Brazil; CHN= China; KP = North Korea; COL = Colombia; EC = Ecuador; FR = France; GER = Germany; IND = India; THA = Thailand and UK = United Kingdom.

Fig. 2. Árbol filogenético de la región ITS-5.8S que indica el posicionamiento de *Agaricus* sp. (negrilla) dentro del clado *Agaricus subrufescens*. Valores de Bootstrap sobre los nodos corresponden a Máxima verosimilitud y Vecino más cercano respectivamente. Enraizamiento en punto medio. Barra = número de sustituciones por posición. BR = Brasil; CHN= China; KP = Corea del Norte; COL = Colombia; EC = Ecuador; FR = Francia; GER = Alemania; IND = India; THA = Tailandia y UK = Reino Unido.

Taxonomy

Agaricus subrufescens Peck, Ann.

Rep. Reg. N.Y. St. Mus. 46: 105 (1894).

Figs. 3 A-K

Basidiome agaricoid, solitary with a slender, elongated stipe and a convex whitish-yellowish pileus with slightly reddish fine-grained scales. Growing on soil with abundant humus-rich mantle and leaf litter. **Pileus** 8–9 cm in diameter; convex with a tendency to flatten according to development, slightly scaly and floccose, yellowish-white (#d8d6cf) to reddish in colour,

more concentrated in the central part of the pileus (#b5948e). **Lamel-
lae** free, strongly narrowed, pinkish (#bb9b90) when young and brown
(#79615b) when come old. **Stipe** 10 cm long and 1 cm wide; ribbed sub-
cylindrical; smooth and white, with bulbous base 1.30 cm wide generated
from abundant mycelium on the substrate. Upper ring attached to the hy-
menium, with hanging white membranous hymenial veil, which is smooth
upper side and squamulose lower side. **Sporeprint** dark brown (#877459).

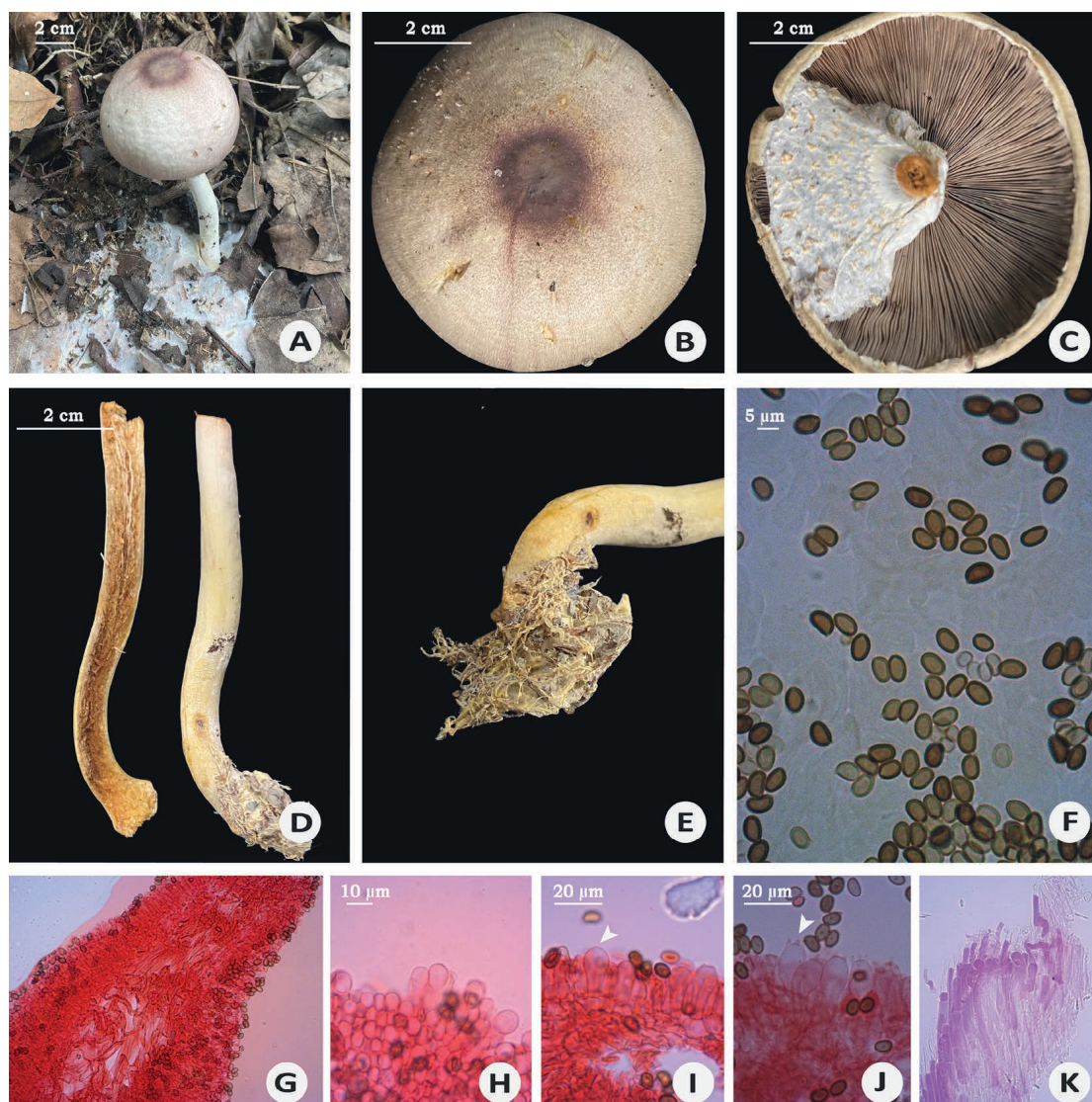


Fig. 3. *Agaricus subrufescens*. Macroscopy: A) General view *in situ* with abundant mycelium on the substrate. B) Surface of the pileus. C) Lamellae. D) Stipite. E) Rhizomorph at the base of stipite. Microscopy: F) Basidiospores. (G) Interwoven trama in the hymenium. (H) Cheilocystidia. (I-J) Basidia. (K) Hyphal system in the stipitipellis. CRUZ, D., BP001 (HUTPL-2047).

Fig. 3. *Agaricus subrufescens*. Macroscopía: A) Vista general *in situ* con abundante micelio en el sustrato. B) Superficie del píleo. C) Laminillas. D) Estipe. E) Rizomorfo en la base del estipe. Microscopía: F) Basidiosporas. G) Trama entrelazada en el himenio. H) Queilocistidia. (I-J) Basidia. K) Sistema hifal en los estipitipellis. CRUZ, D., BP001 (HUTPL-2047).

Macrochemical reactions.— Schäffer reaction not tested, KOH 10% reaction positive (yellowish).

Basidiospores.— $5.5\text{--}6.5 \times 3.5\text{--}4.5 \mu\text{m}$, $Q = 1.5$, elliptical to suboblong with thick-walled; light brown (#a49074) to dark brown (#877459). **Basidia** $13.5\text{--}16 \times 5.6\text{--}7 \mu\text{m}$; clavate; tetrasporic; thin-walled; hyaline. **Cheilocystidia** $7\text{--}12 \times 6\text{--}10 \mu\text{m}$; subglobose; simple or catenulate; hyaline; thin-walled; frequent to abundant. **Pleurocystidia** not observed. **Pileipellis** cylindrical hyphae $3\text{--}12 \mu\text{m}$ diam.

Geographical distribution.— Usually reported to (sub) tropical broadleaf cloud forests Medel-Ortiz *et al.* (2022) in América, but also reported in Europa and other continents (Table 1). Here is the first record for Ecuador and El Oro-Loja province.

Specimen examined.— ECUADOR. El Oro-Loja; Puyango-Alamor; from outer limit Puyango Petrified Forest; Saprotrophic on humus-rich mantle and leaf litter, 29-May-2021, Cruz, D., BP001, HUTPL(F)2047.

Remarks.— The studied specimen *Agaricus subrufescens* BP001 HUTPL(F)2047 macromorphologically agree with the description of the species reported from Mexico (Velázquez-Narváez *et al.*, 2018) and Dominican Republic (Medel-Ortiz *et al.*, 2022). Microscopically, the studied specimen presents, and elliptical to suboblong basidiospores: $5.5\text{--}6.5 \times 3.5\text{--}4.5 \mu\text{m}$, slightly different from *A. subrufescens* described by (Peck, 1893; in the protologue) which registers a pileus of 5–8 cm diameter, a stipe 5–15 cm long and 0.8 cm wide in Peck and brown elliptic basidiospores of approximately $6\text{--}7 \mu\text{m} \times 4\text{--}5 \mu\text{m}$. Our specimen is more similar to the specimen described from Mexico by Velázquez-Narváez *et al.* (2018) specially for their basidiospore $5.6\text{--}6.9$ (7.5) $\times$ $3.5\text{--}4.6$ (4.9) μm , and also similar to the basidiospores measurements record for *A. fiardii* $4.7\text{--}6 \times 3.2\text{--}4.5 \mu\text{m}$ (Medel-Ortiz *et al.*, 2022).

DISCUSSION

The morpho-molecular analysis carried out in this study confirms the assignment of our specimen as *Agaricus subrufescens*, this being the first time that its presence has been reported in Ecuador, which expands the knowledge about its distribution. The specimen (Fig. 3), was found on the outer perimeter of the Puyango Petrified Forest (PPF) which is into a natural tropical dry forest, characterized by remnant endemic vegetation and an ecosystem of petrified trunks (Jumbo-Eras *et al.*, 2021).

Molecular phylogenetic inference

Phylogenetically our sequence (ON799184) of *Agaricus subrufescens* is cluster together several sequences: *A. blazei* from North Korea (KP) AF161013

and Brazil (BR) AY484697; *A. subrufescens* from Brazil (BR) KJ541796; *A. rufotegulis* from United Kingdom (UK) AY818649; *A. fiardii* from France (FR) JF797201; and *Agaricus* sp. JF514529 of Thailand (THA) in one clade with 99% support in the Neighbor-Joining (NJ) and Maximum Likelihood (ML) analyses (Fig. 2). These taxonomic relationships have been widely discussed by Kerrigan (2005) and Medel-Ortiz *et al.* (2022).

The clade formed with sequences mostly assigned to *A. subrufescens* (Fig. 2) and other *Agaricus* species, was considered as species complex clade “*A. subrufescens*” by Zhao *et al.* (2011). This clade (Fig. 2) includes a sequence obtained from a basidiome identified as *Agaricus fiardii* voucher F2285 LIP Martinique France, (GenBank accession number JF514529) (Zhao *et al.*, 2011). This species *A. fiardii* was originally described from Martinique France by Pegler (1983) and it is morphologically like *A. subrufescens*. Additionally when compare phylogenetically the ITS2 sequences, one clade is generated (data do not show) clustering sequences from *A. fiardii* type material [K(W)234316], and sequences to *A. brasiliensis*; *A. blazei*; and others (Table 1) which are considered synonyms in many studies (Wasser *et al.*, 2002; Kerrigan, 2005; Thongklang *et al.*, 2016; Zied *et al.*, 2021; Medel-Ortiz *et al.*, 2022).

Surprisingly one sequence named as *Agaricus campestris* voucher NVE470, (GenBank accession number KF937296) isolated from basidiome is clustered into this clade. This basidiome was found in Boyacá-Arcabuco, Colombia and referenced according to GenBank information to the work of Vasco-Palacios *et al.* (2014). However, in Vasco-Palacios *et al.* (2014) no morphological information or sequence analysis is presented for this specimen. So that, in the present study this sequence and species are considered as a probable misassignment.

Agaricus subrufescens, has been widely discussed at taxonomic level (Wasser *et al.*, 2002; Kerrigan, 2005; Thongklang *et al.*, 2016; Zied *et al.*, 2021; Medel-Ortiz *et al.*, 2022), and is considered as an important edible fungus with medicinal properties (Hetland *et al.*, 2011). Their efficiency in the immune system against allergies, tumours and other health problems is attributed to the generation of secondary metabolites mainly β -glucan polysaccharides (Hetland *et al.*, 2011). Therefore, this fungus is commercially cultivated in different American, European countries and other continents (Wisitrassameewong *et al.*, 2012; Thongklang *et al.*, 2016; Velázquez-Narváez *et al.*, 2018; Zied *et al.*, 2021).

This finding strengthens Ecuador as Megadiverse, with fungi in tropical areas with edible potential, unlike other fungi that are mainly known for high Andean areas used for indigenous communities (Andrade *et al.*, 2012; Gamboa-Trujillo *et al.*, 2019). Therefore, further exploration and knowledge of fungal diversity is needed, and it should also be included in conservation policies, as is currently the case for endemic or endangered species of flora and fauna (Albán, 2010). These new non-timber forest resources have potential to be exploited biotechnologically.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

- Albán, A. (2010). Política y Estrategia Nacional de Biodiversidad del Ecuador. *Ministerio Del Ambiente*: 2-5.
- Andrade, J., Malagón, O., Piepenbring, M. & Armijos, C. (2012). Etnomycología y valor nutricional de macrohongos silvestres de la comunidad indígena saraguro en el sur del Ecuador. *Boletín de la Sociedad Micológica Madrid* 36: 193-202.
- Batallas-Molina, R., Moya-Marcalla, G. F. & Navas Muñoz, D. (2021). Listado de la colección de hongos (Ascomycota y Basidiomycota) del Herbario Nacional del Ecuador (QCNE) del Instituto Nacional de Biodiversidad (INABIO). *Avances en ciencias e ingenierías* 12 (20): 38-71. <https://doi.org/10.18272/aci.v12i1.1755>
- Benítez, Á., Cruz, D., Vega, M., González, L., Jaramillo, N., López, F. & Aguirre, Z. (2021). Briófitos y hongos (liquenizados y no liquenizados) del Parque Universitario Francisco Vivar Castro, Loja, Ecuador. *Bosques Latitud Cero* 11 (2): 1-18. <https://doi.org/10.54753/blc.v11i2.1102>
- Cao, B., Mao-Qiang, He, Zhi-Lin, L., Ming-Zhe, Z., Sheng-Long, W. & Rui-Lin, Z. (2021). A revision of *Agaricus* section *Arvenses* with nine new species from China. *Mycologia* 113 (1): 191-211. <https://doi.org/10.1080/00275514.2020.1830247>
- Cruz, D. & Masache, D. (2023). Listado y guía visual preliminar de macrohongos del Bosque Petrificado de Puyango-Ecuador. *ACI Avances en Ciencias e Ingenierías* 15 (2). <https://doi.org/10.18272/aci.v15i2.2939>
- Cruz, D., Suárez, J. P. & Piepenbring, M. (2016). Morphological revision of Tulasnellaceae, with two new species of *Tulasnella* and new records of *Tulasnella* spp. for Ecuador. *Nova Hedwigia* 102 (3-4): 279-338. <https://doi.org/10.13140/RG.2.1.2185.1604>
- Ehrich, J. & Hutter, S. (2020). Der Mandel-Egerling *Agaricus subrufescens* - ein weiterer Neomycet für Deutschland. *Boletus* 41 (1): 17-25.
- Gamboa-Trujillo, P., Wartchow, F., Cerón-Martínez, C., Andi, D., Uwinjin, P., Grefa, G., ... & Piyaguaje, N. (2019). Edible Mushrooms of Ecuador: consumption, myths and implications for conservation. *Ethnobotany*

- Research and Applications* 18: 1-15. <https://ethnobotanyjournal.org/index.php/era/article/view/1617>
- Gates, G. M., Goyes, P., Gundogdu, F., Cruz, J. & Ratkowsky, D. A. (2021). Small plot surveying reveals high fungal diversity in the Ecuadorian Amazon – a case study. *Current Research in Environmental & Applied Mycology* 11 (1): 16-36. <https://doi.org/10.5943/cream/11/1/>
- González, M. R., Figlas, D. & Curvetto, N. (2011). *Agaricus blazei* production on non-composted substrates based on sunflower seed hulls and spent oyster mushroom substrate. *World journal of microbiology & biotechnology* 27 (6): 1331-1339. <https://doi.org/10.1007/s11274-010-0582-5>
- Hetland, G., Johnson, E., Lyberg, T. & Kvalheim, G. (2011). The mushroom *Agaricus blazei* Murill elicits medicinal effects on tumor, infection, allergy, and inflammation through its modulation of innate immunity and amelioration of Th1/Th2 imbalance and inflammation. *Advances in Pharmacological Sciences* 2011: 1-10. <https://doi.org/10.1155/2011/157015>
- Hidalgo, V. G. V. & López, H. O. D. (2019) *Evaluación de las actividades biológicas de compuestos bioactivos del hongo Agaricus blazei*. Universidad Técnica de Ambato. Facultad de Ciencia e Ingeniería en Alimentos. Carrera de Ingeniería Bioquímica. <http://repositorio.uta.edu.ec/jspui/handle/123456789/29127>
- Jumbo-Eras, E., Herrera-Feijoo, R. J., Ávila-Andrade, A., Chicaiza-Ortiz, Á., Morocho-Cuenca, M. & Chicaiza-Ortiz, C. (2021). Evaluación de la biodiversidad, amenazas y estatus de conservación de la flora y fauna del Bosque Petrificado Puyango. *Green World Journal* 4 (2): 1-9. <https://doi.org/10.53313/GWJ42018>
- Katoh, K., Rozewicki, J. & Yamada, K. D. (2019). MAFFT online service: multiple sequence alignment, interactive sequence choice and visualization. *Briefings in bioinformatics* 20 (4): 1160-1166. <https://doi.org/10.1093/bib/bbx108>
- Kerrigan, R. W. (2005). *Agaricus subrufescens*, a cultivated edible and medicinal mushroom, and its synonyms. *Mycologia* 97 (1): 12-24. <https://doi.org/10.3852/MYCOLOGIA.97.1.12>
- Læssøe, T. & Petersen, J. H. (2008). Svampelivet på ækvator. Hongos de Ecuador. *Svampe* 58: 1-53.
- Largent, D. L., Johnson, D. & Watling, R. (1973). *How to identify mushrooms to genus III: Microscopic features*. Eureka, CA: Mad River Press. 148 pp. <https://doi.org/10.2307/2484126>
- Mata, G. & Savoie, J. M. (2012) *Agaricus subrufescens* un hongo comestible y medicinal de gran potencial en México. Hongos comestibles y medicinales en Iberoamérica: investigación y desarrollo en un entorno multicultural. Chiapas, México. El Colegio de la Frontera Sur. p. 137-144.
- Medel-Ortiz, R., Garibay-Orijel, R., Argüelles-Moyao, A., Mata, G., Kerrigan, R. W., Bessette, A. E., Geml, J., Angelini, C., Parra, L. A. & Chen, J. (2022). *Agaricus macrochlamys*, a new species from the (sub)tropical Cloud Forests of North America and the Caribbean, and *Agaricus fi-*

- ardii*, a new synonym of *Agaricus subrufescens*. *Journal of Fungi* 8: 664. <https://doi.org/10.3390/jof8070664>
- Morante-Carballo, F., Herrera-Narváez, G., Jiménez-Orellana, N. & Carrión-Mero, P. (2020). Puyango, Ecuador petrified forest, a geological heritage of the Cretaceous Albian-middle, and its relevance for the sustainable development of geotourism. *Sustainability* 12 (16): 6579. <https://doi.org/10.3390/su12166579>
- Ordoñez, M. E. (2018). *Fungi del Ecuador*. Versión 2018.0. Fungario QCAM, Pontificia Universidad Católica del Ecuador. <https://bioweb.bio/fungi-web>
- Peck, CH. (1893). New York State. *Mushroom Annual Report* 46: 83-152.
- Pegler, D. N. (1983). Agaric flora of the Lesser Antilles. *Kew Bulletin, Additional Series* 9: 1-667.
- Sánchez, J. E. & Mata, G. (2012). Cultivo y aprovechamiento de macrohongos. Una tendencia global en crecimiento. In Mata G, JE Sánchez eds. Hongos comestibles y medicinales en Iberoamérica: investigación y desarrollo en un entorno multicultural. Chiapas, México. El Colegio de la Frontera Sur. p. 365-376.
- Stecher, G., Tamura, K. & Kumar, S. (2020). Molecular Evolutionary Genetics Analysis (MEGA) for macOS. *Molecular Biology and Evolution* 37 (4): 1237-1239. <https://doi.org/10.1093/MOLBEV/MSZ312>
- Thongklang, N., Chen, J., Bandara, A. R., Hyde, K. D., Raspé, O., Parra, L. A. & Callac, P. (2016). Studies on *Agaricus subtilis*, a new cultivatable species from Thailand, incidentally reveal the presence of *Agaricus subrufescens* in Africa. *Mycoscience* 57 (4): 239-250.
- Toapanta-Alban, C. E., Ordoñez, M. E., Barnes, C. W. & Blanchette, R. A. (2021). Taxonomy of the major rhizomorphic species of the “*Melanopus* group” within Polyporaceae in Yasuní National Park, Ecuador. *Plos one* 16 (8): e0254567. <https://doi.org/10.1371/journal.pone.0254567>
- United Nations Environment Programme (2014). Definición de países megadiversos, Biodiversidad A-Z. <https://www.biodiversitya-z.org/content/megadiverse-countries>
- Vasco-Palacios, A. M., López-Quintero, C., Franco-Molano, A. E. & Boekhout, T. (2014). *Austroboletus amazonicus* sp. nov. and *Fistulinella campinaranae* var. *scrobiculata*, two commonly occurring boletes from a forest dominated by *Pseudomonotes tropenbosii* (Dipterocarpaceae) in Colombian Amazonia. *Mycologia* 106 (5): 1004-1014. <https://doi.org/10.3852/13-324>
- Velázquez-Narváez, A. C., Medel, R., Mata, G. & Espinosa de los Monteros, A. (2018). *Agaricus subrufescens* en México: un recurso forestal no maderable, comestible, medicinal y potencialmente cultivable. *Madera y Bosques* 24 (2): 1-13. <https://doi.org/10.21829/myb.2018.2421574>
- Wasser, S. P., Didukh, M. Y., de Amazonas, M. A. L., Nevo, E., Stamets, P. & da Eira, A. F. (2002). Is a widely cultivated culinary- medicinal Royal Sun *Agaricus* (the Himematsutake Mushroom) indeed *Agaricus*

- blazei* Murrill?. *International Journal of Medicinal Mushrooms* 4: 267-290. <https://doi.org/10.1615/IntJMedMushr.v4.i4.10>
- White, T. J., Bruns, T., Lee, S. J. W. T. & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR protocols: a guide to methods and applications* 18 (1): 315-322.
- Wisitrassameewong, K., Karunarathna, S. C., Thongklang, N., Zhao, R., Callac, P., Moukha, S., Férandon, C., Chukeatirote, E. & Hyde, K. D. (2012). *Agaricus subrufescens*: A review. *Saudi Journal of Biological Sciences* 19 (2): 131-146. <https://doi.org/10.1016/J.SJBS.2012.01.003>
- Zhao, R., Karunarathna, S., Raspé, O., Parra, L. A., Guinberteau, J., Moinard, M., De Kesel, A., Barroso, G., Courtecuisse, R., Hyde, K. D., Guelly, A. K., Desjardin, D. E. & Callac, P. (2011). Major clades in tropical *Agaricus*. *Fungal Diversity* 51: 279-296.
- Zied, D. C., Vieira Junior, W. G., Soares, D. M. M., Stevani, C. V., Dias, E. S., Iossi, M. R. & Pardo-Giménez, A. (2021). Overview of four *Agaricus subrufescens* strains used in the last 15 years in Brazil and other countries and current potential materials for the future. *Mycological Progress* 20 (8): 953-966. <https://doi.org/10.1007/s11557-021-01711-x>