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Cortinarius mahiquesii, a new subhypogeous species from Catalonia (Iberian Peninsula)

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Key words

Cistus community
Cortinariaceae
Cortinarius
internal transcribed spacer (ITS)
Mediterranean area
taxonomy

Abstract We describe and comment on a new species of *Cortinarius* (Basidiomycota), *C. mahiquesii*, collected in a pure *Cistus monspeliensis* scrub community in Catalonia (NE of the Iberian Peninsula). Drawings of microscopic characters, scanning microphotographs of the basidiospores, and colour pictures of the basidiomes in their natural habitat are provided. A molecular analysis was carried out to characterize the new species using ITS sequences.

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INTRODUCTION

Cortinarius belongs, together with *Entoloma*, to the larger agaric genera (Noordeloos 2004, Peintner et al. 2004). Although these genera are fairly well known, numerous new species are still to be expected, not only from extra-European areas (e.g. Peintner et al. 2003, Manimohan et al. 2006, Gates & Noordeloos 2007), but also from Europe.

In recent years, numerous papers, including both evolutionary and taxonomic studies, have been published about the genus *Cortinarius* (subgenus *Phlegmacium*) in Europe (Garnica et al. 2003, 2005, Frøslev et al. 2005, 2006, 2007, Bidaud et al. 2008, Ortega et al. 2008). In spite of this extensive research into European *Cortinarius*, the description of new species is quite frequent, especially in certain Mediterranean ecosystems that were poorly studied in the past. This is the case of the *Cistus* scrub communities in north-eastern Spain (Catalonia), where recent studies have shown their very particular mycobiota (especially mycorrhizal macrofungi) and have led to the description of several new taxa (Vila & Llimona 2002, 2006; Ballarà et al. in press): *C. aureocistophilus*, *C. llimonae*, *C. xanthosarx*, etc.

In this paper we propose a new species, *Cortinarius mahiquesii*, collected in the Alt Empordà (Girona) in a pure *Cistus monspeliensis* community. The characterization of *C. mahiquesii* is based on both morphological and molecular (ITS) data. It is most characterized by the following salient features: i) stocky basidiomes; ii) subhypogeous development; iii) a grey-olivaceous coloured pileus when mature; iv) annular-cortina on the stipe; v) a farinaceous smell; vi) a cutis-structure pileipellis with a tendency to form a trichoderm. Due to its molecular and morphological characters, *C. mahiquesii* should be included in the Glaucopodes clade (sensu Frøslev et al. 2005) and in sect. *Glaucopodes*.

MATERIAL AND METHODS

The macromorphological study was carried out on both young and mature fresh specimens. For the macrochemical test, we used KOH 15 – 40 % solution on cap, bulb and context. In order to define the basidiome habit, we used the following parameters: slenderness index (Is = l^2/Dxd; l = stipe length, D = pileus diam, d = stipe width; according to Kuyper 1986); pileus diam/stipe length (Pd/Sl); stipe length/stipe width (Sl/Sw) and stipe width/bulb width (Sw/Bw). The micromorphological analysis was performed using free-hand radial sections of pileus and longitudinal sections of the gills mounted in KOH (3 %) and Congo red (in NH_4OH). Twenty spore measurements of several basidiomes were made and the minimum, mean and maximum values, as well as the length/width ratio (Q = L/w) were calculated. The spore ornamentation was recorded using a Field Emission Scanning Electron Microscope Leo (Zeiss), model 1539 Geminis (FESEM). The material studied is housed in the herbaria GDA (University of Granada, Spain) and JVG (personal herbarium of the first author). The classification of *Cortinarius* follows Brandrud et al. (1994), Frøslev et al. (2005), Garnica et al. (2005), Consiglio et al. (2007b), and Bidaud et al. (2008).

For the molecular analysis, the total genomic DNA was extracted using the CTAB method (Doyle & Doyle 1987). The entire ITS region (ITS-1, 5.8S and ITS-2) was amplified by PCR, using primers ITS-5 (White et al. 1990) and C26A (Wen & Zimmer 1996). The PCR reactions were performed in a volume of 50 µl under standard conditions (Innis et al. 1990).

The ITS region was amplified for *C. mahiquesii* and its most closely related species (taken from GenBank). The selection of sequences taken from the GenBank was based on the following: the morphological analyses carried out by us; the results of Garnica et al. (2003, 2005) and Frøslev et al. (2005); and BLAST searches. The EMBL accession number for *C. mahiquesii* is included in the Specimens examined section, and the GenBank accession number for the remaining sequences are shown in the ITS tree. The ITS sequences were aligned using the CLUSTAL option of the MEGALIN program from the DNA-

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star software package (LASERGEN), followed by a visual inspection. The genetic distances (uncorrected *p*-distance) between the sequences were calculated using MEGA version 4 (Tamura et al. 2007).

Phylogenetic analysis was performed using maximum likelihood (ML) criterion, as implemented in PAUP* 4.0b10 (Swofford 2003). The best-fit model of nucleotide substitution for the ITS data was Felsenstein 81 (Felsenstein 1981) with variable sites following a discrete gamma distribution (F81 + G), as implemented in Modeltest 3.06 (Posada & Crandall 1998). ML searches were implemented with a starting tree obtained via Neighbor Joining (NJ) and the Jukes-Cantor model, and using tree bisection-reconnection (TBR) branch-swapping. Gaps were treated as missing data. Bootstrap values were calculated to assess branch support in the resulting trees. These analyses were performed using 1 000 pseudoreplicates and TBR branch swapping beginning with the topology resulting from the NJ analysis. Fig. 1 shows the maximum likelihood tree showing the relationships between *C. mahiquesii* and its most closely related species.

Taxonomy

Cortinarius mahiquesii Vila, A. Ortega & Suár.-Sant., *sp. nov.* — MycoBank MB512434; Fig. 2–4

Basidiomata subhypogea. Pileus usque 40 mm in diam, convexo-applanatus, griseus vel griseo-azureus, saepe leviter olivaceus, per aetatem luteo-maculatus; superficies viscida, deinde sicca, ut videtur leviter velutina. Lamellae primo azureae, per maturitatem brunneo-griseae. Stipes usque 30 × 11 mm, bulbo debiliter marginato, prominenti aut mediocri, munitus; primo azureus, deinde albescens coloratus; siccus; anullaris zona bene delimitata; per aetatem lutescens. Caro a farina odorus. Sporae 9.8–12 × 5–6.1(–6.4) µm, amydaloides, subcitriformes vel citriformes (Q: L/w = 1.86–2.1), leviter usque aliquantum valde verrucosae, dense connexis tuberculis obtectae. Acierum sterilia cellulae abundantes, 20–25 × 6.5–9.5 µm, cylindricae, clavatae, subutriformes vel subpyriformes. Pileipellis duplex; epicutis a numerosis, septatis, crassitunicatis, 80–180 µm longis, apicalibus elementibus composita. — Holotypus (hic designatus) prope urbem el Port de la Selva dictam, in Catalonia (Hispania), a J. Vila et X. Llimona lectus, 18-1-2008, 240 m altitudinis, sub *Cistus monspeliensis*, in herb. GDA 54298 conservatus.

Etymology. This species is dedicated to Rafael Mahiques Santandreu (Quatretonda), due to his significant contribution to the study of the genus *Cortinarius* in the Iberian Peninsula.

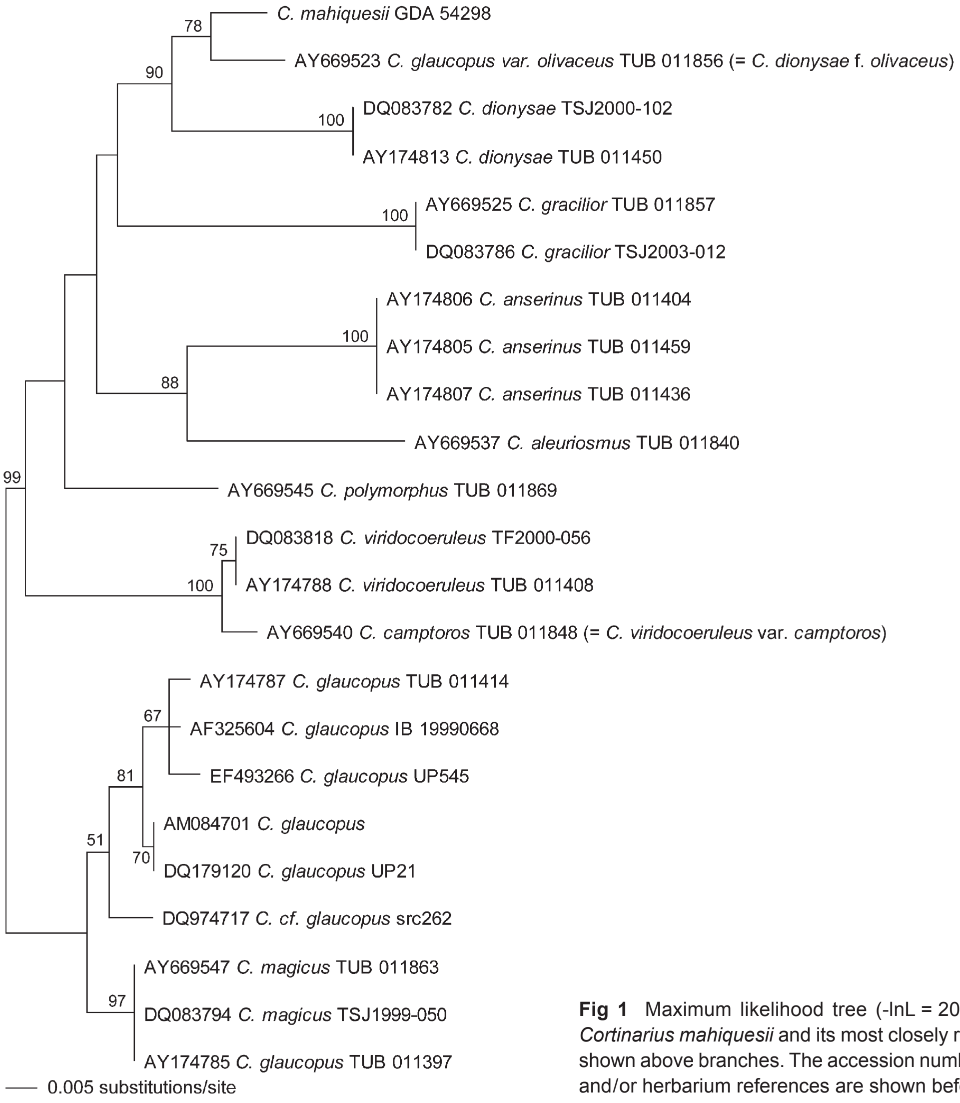

Fig 1 Maximum likelihood tree (-lnL = 2099.12) showing the relationships between *Cortinarius mahiquesii* and its most closely related species. Bootstrap values ≥ 50 % are shown above branches. The accession number (for the sequences taken from GenBank) and/or herbarium references are shown before and after each taxon name respectively.

Basidiomes subhypogeous, isolate, gregarious or forming small clusters, stocky habit (Is = 1.13–1.44–1.78). *Pileus* up to 40 mm in diam, convex or hemispheric, later convex-flattened, rarely slightly depressed towards the centre; firstly bluish, mainly at the margin and in specimens that are still buried, later greyish, bluish grey or brown-grey, with olivaceous hues, which intensify in mature basidiomes; in older specimens, the pileus is intensely yellowing or brown-yellow all over. The surface is glutinous, mainly when young, although it soon dries out; typically, it has abundant adhered earth particles, as the pileus surface acquires a fibrous-downy or fibrous-velvety look during the fruiting process, mainly in the disc, and hardy fibrous towards the margin. Margin involute, partially straight in mature specimens, slightly undulated in adult basidiomes. *Lamellae* adnate or sinuate, dense, rather thin, sometimes bifurcate, firstly bluish, later becoming bluish grey, grey-beige and, finally, grey-brown; edge paler and slightly irregular; lamellulae abundant (1 : 1 to 1 : 3). *Spore print* ferruginous. *Stipe* up to 30 × 11 mm, shorter than the pileus diameter (Pd/Sl = 1.43–1.53–1.68), stocky to moderately slender (Pl/Pw = 1.4–2.02–2.67) with a poorly marginate, prominent to moderate bulb (Sw/Bw = 0.52–0.62–0.68) up to 17 mm in diam; bluish when young, rather intense later, when maturing, whitish or slightly greyish; surface dry, with thin fibrilles at the apex and a typical, well-delimited annular zone, which forms a faint circular line around the stipe; with whitish mycelial strands; when specimens become older, the stipe intensely yellowing to yellow-brown, like the pileus. *Context* rather compact, whitish or creamy, with bluish hues at the apical part of the stipe and bluish grey under the pileipellis and above the lamellae, yellowing in the basal part of the bulb; a slightly floury smell, becoming partially raphanoid in older specimens. KOH reaction yellowish, darkening on the context, and dark brown-violaceous on the pileipellis.

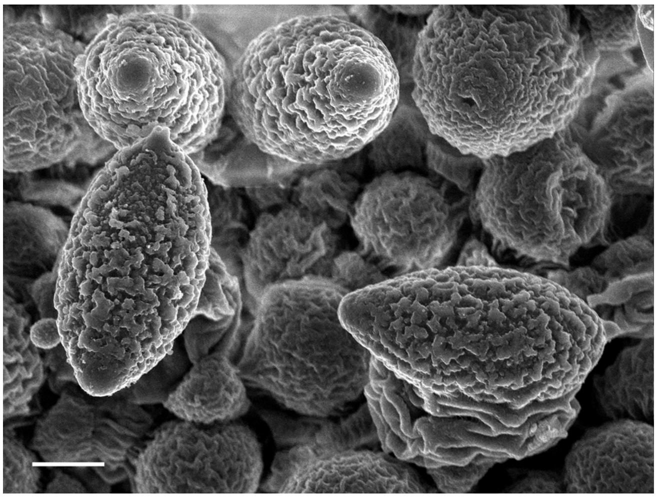

Fig. 2 *Cortinarius mahiquesii* (holotype GDA 54298). Scanning (FESEM) micrographs of the spores. — Scale bar = 3 µm.

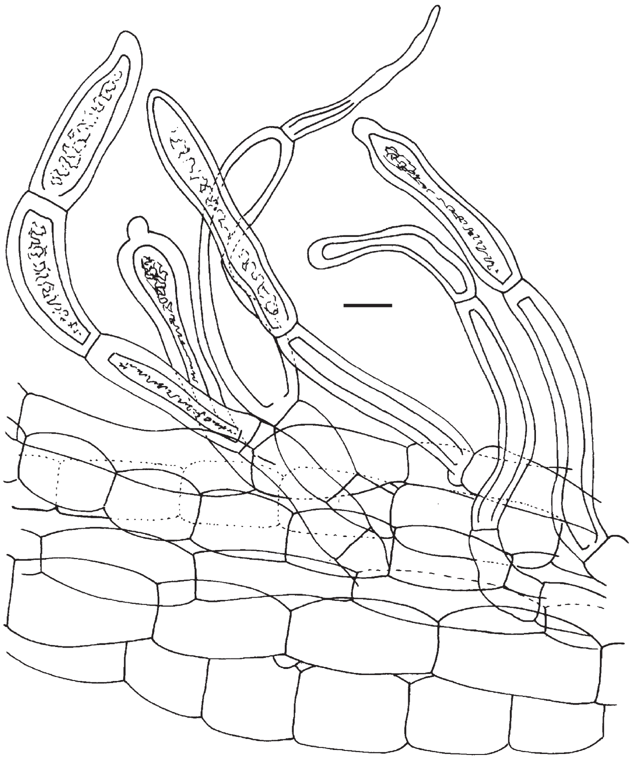

Fig. 4 *Cortinarius mahiquesii* (holotype GDA 54298). Structure of the pileipellis. — Scale bar = 8 µm.

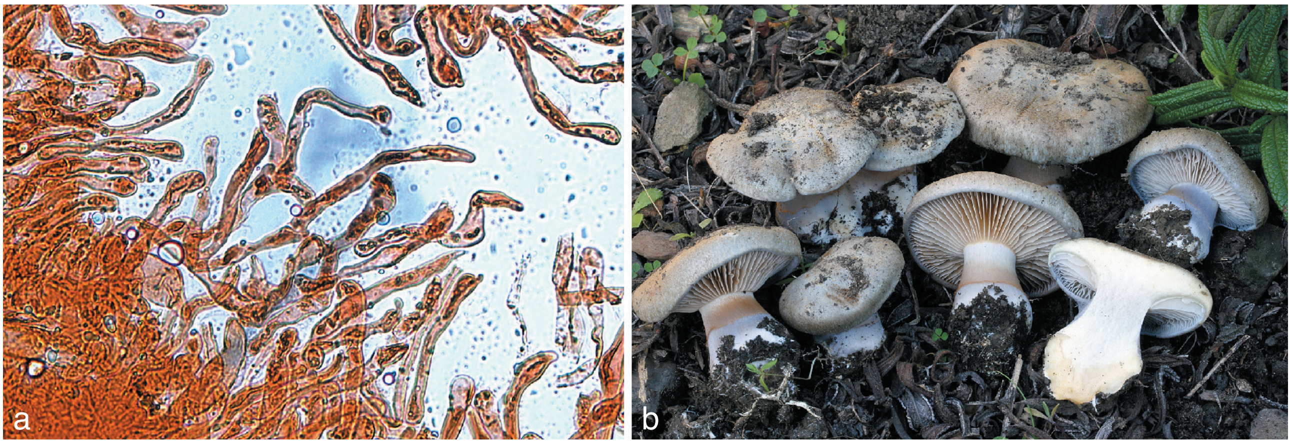

Fig. 3 *Cortinarius mahiquesii* (holotype GDA 54298). a. Terminal elements of epicutis; b. basidiomes in their own habitat.

Spores 9.8–12 × 5–6.1(–6.4) µm, mv: 10.7–11.2 × 5.4–5.8 µm, rather strongly dextrinoid (following Vesterholt 2005), amygdaloid, subcitriform to citriform (Q: L/w = 1.86–2.1; mv: 1.93–1.96), moderately to rather strongly verrucose with ± densely interconnected warts. *Basidia* 25–35 × 7.5–10 µm, 4-spored, cylindrical to clavate, forming a ± dense palisade. *Lamellar edge sterile cells* abundant, 20–25 × 6.5–9.5 µm, cylindrical, clavate, subutrifom or subpyriform. *Subhymenium* differentiated, ramose, made up of jumbled ('puzzle') little cells (c. 1.5 µm wide). *Hymenophore trama* ± parallel, formed by pale, 5–10 µm wide hyphae. *Pileipellis* duplex. *Epicutis* structure a scarcely gelified ixocutis, with tendency to become a trichoderm, formed by flexuous and straight terminal elements, moderately to strongly thick-walled, septate, with a granular greyish pigment, 80–180 µm long, sometimes with enlarged basal cells. The apical elements show a variable shape (cylindrical, claviform or subcapitate), 5–10 µm wide. *Hypocutis* differentiated, with ± parallel or slightly interwoven, up to 10–22 µm wide hyphae with numerous septa, which delimit short cellular elements, disposed in a pseudo-parenchymatous (subcellular) structure.

Specimens examined. Spain, Catalonia, Perafita, el Port de la Selva (Girona), alt. 240 m, subhypogeous, under *Cistus monspeliensis*, on acid soil, 18 January 2008, leg. *J. Vila & X. Llimona*, *GDA 54298* (holotypus, designated here) (EMBL accession number FM202139), isotypus in *JVG 1080118-18*.

Taxonomic notes

On the basis of its morphological features, *C. mahiquesii* belongs to sect. *Glaucopodes*, subsect. *Amoenolentes* (Bidaud et al. 2008), or, according to Consiglio et al. (2007a), is part of subsect. *Magici*. From a molecular point of view, *C. mahiquesii* relates to the Glaucopodes (Garnica et al. 2003, Frøslev et al. 2005) or Caerulescentes (Garnica et al. 2005) clades, and specifically to the *C. dionysae* complex (Fig. 1). The relationship between *C. mahiquesii* and *C. dionysae* is clearly shown by its farinaceous smell and its amygdaloid to citriform spores.

Cortinarius dionysae is a very variable species morphologically, as three colour forms or variants are recognized: *C. dionysae* forma *dionysae*, *C. dionysae* forma *olivaceus*, and *C. dionysae* var. *avellaneus* (Bidaud et al. 2008). This species is widely distributed throughout Europe (Iberian Peninsula, Mahiques 1999, 2001, 2002, 2004, 2006; France, Bidaud et al. 2008; Italy, Consiglio et al. 2005, 2007b; Germany, Garnica et al. 2003, 2005; Frøslev et al. 2005; Nordic countries, Brandrud et al. 1992; Switzerland, Breitenbrach & Kränzlin 2000) and Northern Africa (Malençon & Bertault 1970), producing basidiomes in very diverse habitats and growing both in coniferous forests and broad-leaved forests. In the Mediterranean region, it has been found in calcicolous *Quercus ilex* forests (Malençon & Bertault 1970, Ortega & Mahiques 1995, Bidaud et al. 2008) and silicicolous *Q. suber* forests (Malençon & Bertault 1970, Ortega et al. 1994). These latter collections are biogeographically and ecologically related to *C. mahiquesii*. The specimens of *C. dionysae* collected from *Q. suber* forests in Algérie (Malençon & Bertault 1970: 499–501) are significantly similar to our species in the following aspects: i) gregarious or fasciculate growth; ii) habitat (Mediterranean sclerophyllous communities on siliceous soils); iii) similar basidiome size; iv) abundant edge sterile cells with a similar shape; and v) a subhymenium with a similar ramose structure.

Cortinarius mahiquesii is easily distinguished from *C. dionysae* on the basis of the following characters: i) basidiome with subhypogeous development; ii) exclusive growth in siliceous *Cistus* scrub communities; iii) hardly gelified pileipellis; iv) pileus with grey-olive tinges; v) ephemeral bluish hues present (in young specimens) in the pileus, lamellae and stipe, rarely in the context; vi) epicutis with a tendency to a trichodermis, whose hyphae present numerous end-free, terminal differentiated elements (similar to hairs), thick-walled, with variable apical cells. The structure of this epicutis is not present in any of the related species.

From a molecular point of view, *C. mahiquesii* is nested in the *C. dionysae* clade, forming a strongly supported clade with *C. glaucopus* var. *olivaceus* (= *C. dionysae* forma *olivaceus*) (Fig. 1). However, the genetic distance between *C. mahiquesii* and *C. dionysae* forma *olivaceus* (2.2 %) shows the specific differentiation of these species. The genetic distances between *C. mahiquesii* and the other *C. dionysae* collections are higher (4 %). The high genetic distance between *C. dionysae* forma *olivaceus* and the other two collections of *C. dionysae* (4 %) is notable and in contrast to the absence of genetic distance between these two latter collections (p = 0.000). This fact could support the recognition of *C. dionysae* forma *olivaceus* as a different species from *C. dionysae*. However, a more detailed study is needed to confirm this.

Other related taxa present in the Mediterranean region, and especially in Spain (Mahiques 2006) are *Cortinarius anserinus* (= *C. amoenolens*) and *C. viridocoeruleus*. The separation of these species from *C. mahiquesii* can clearly be seen in the basidiome morphology and the lack of a farinaceous smell. *Cortinarius anserinus* is distinguished by its i) epigeous growth; ii) different habitat (nemoral broad-leaved forests); iii) faint, pleasant-sweetish, not farinaceous smell; and iv) bitter taste of the cap cuticle. With regards to the second species, mycologists (Bidaud et al. 2008) currently distinguish two ecological variants: a) *C. viridocoeruleus* var. *camptoros* growing in coniferous forests; and b) *C. viridocoeruleus* var. *viridocoeruleus*, fruiting in the warm *Quercus* forests and more prevalent in the Iberian Peninsula than the former variety (Mahiques 2006). Consiglio et al. (2007a) also distinguished *C. viridocoeruleus* var. *pervelatus*. The morphological separation of *C. viridocoeruleus* s.l. from *C. mahiquesii* is based on the following characters: i) epigeous growth; ii) a different habitat (nemoral or sclerophyllous broad-leaved or coniferous woodlands); iii) a variable, not farinaceous smell; and iv) smaller spores (i.e. 9.2–10.4 × 5.4–6 µm, Consiglio et al. 2007a). Fig. 1 shows the clear differentiation of *C. mahiquesii* from *C. anserinus* and *C. viridocoeruleus* s.l. from a molecular point of view, since the mean genetic distances between these species are 5.6 % and 5.4 %, respectively.

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