



## ***Albomagister virgineus* sp. nov., a second species of *Albomagister* in Europe**

**Corriol G<sup>1</sup> and Jargeat P<sup>2</sup>**

<sup>1</sup> Conservatoire botanique national des Pyrénées et de Midi-Pyrénées. (National botanical conservatory for Pyrenees and Midi-Pyrénées region of France and BBF Herbarium). Vallon de Salut. B.P. 315. 65203 Bagnères-de-Bigorre Cedex. France.

<sup>2</sup> Université Paul Sabatier - Toulouse 3, CNRS, ENSFEA, IRD. Laboratoire Evolution et Diversité Biologique UMR5174 EDB. 118 route de Narbonne. 31062 Toulouse Cedex. France

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### **Abstract**

*Albomagister virgineus* is described as a new species from a collection from Hautes-Pyrénées in France. Morphological and molecular studies clearly separate it from related species of *Albomagister*. The assignment to *Albomagister* is presently suitable, however, morphological characters and a complementary morphological analysis of *A. alesandrii*, the only representative of the genus in Europe, contradicted the recent circumscription of this genus. Molecular results confirmed these findings and suggest heterogeneity among the *Albomagister*.

**Key words** – 1 new species – Basidiomycota – France – Hautes-Pyrénées – Taxonomy – Tricholomataceae

### **Introduction**

In the field, this tiny inocyboid and seemingly white-spored collection nestled in a thick boxwood litter, a peculiar ecology, could not be assigned to any known genus of us.

In laboratory, a detailed anatomical study confirmed this first impression: not only was it impossible for us to relate it to a known species, but we also had difficulties in finding the corresponding genus. The only hypothesis we had contemplated on-site (*Pseudobaeospora*) was contradicted by microscopical observations: spores were too large, non dextrinoid and there were no sclerobasidia.

Therefore we archived our description and waited for an opportunity for further determination. Finally, eight years later, a new small mushroom collected in Corsica was described (Moreau et al. 2015). It belonged to the *Albomagister*, a freshly described Tricholomataceae (Sanchez-Garcia et al. 2014). It reminded us of our description, and we thought that it might fit in this genus. Therefore we performed a molecular analysis to confirm that our mushroom belonged to the *Albomagister*.

## Materials & Methods

### Morphological description

Basidiomata were collected on November 2007, in the vicinity of Bagnères-de-Bigorre (Hautes Pyrénées, France), and were fully described on the fresh specimens and photographed *in situ*. Colours were coded based on Munsell Soil Color Charts (2000). Microscopy observations were performed on fresh material in water, in a 5 % KOH solution, Congo red, Melzer's reagent, lactic cotton blue, Crésyl blue, and on dry material in Cléménçon's GSM and Melzer's reagent. Spore measurements, made on N spores observed on spore prints rehydrated in Cléménçon's GSM, are presented according to the D1, 9 format:

[N] (mini) d1-*median*-d9 (maxi), where d1 and d9 are the 1<sup>st</sup> et 9<sup>th</sup> deciles (Fannechère 2005). The length/width rate (Q) was recorded following the same rules. As for the other elements, only minimum and maximum dimensions were indicated. The scale bars on the microscopy picture chart represent ten micrometers.

Specimens collected in 2001, coming from a boxwood (*Buxus sempervirens* L.) shrub located in Courcelles-les-Quingey (Doubs, France), and archived as "*Pseudobaeospora* sp. undet." were also studied (herb. GC01101108, Fig. 6).

### Molecular analysis

One basidiome from the November 2007 (GC 07110503) collection, coded GC01, one specimen from the "*Pseudobaeospora* sp. undet." 2001 collection (GC01101108, Courcelles-les-Quingey, Doubs, France), coded GC02, and 3 basidiomata, initially attributed to *Pseudobaeospora* aff. *paulochroma* Bas, collected by Jean-Marc Moingeon in Courcelles-les-Quingey (Doubs, France) in 2011, coded JMM01 to JMM03, were analyzed. *Pseudobaeospora* aff. *paulochroma* was illustrated and described by Moyne et al. (2007). Photographs of JMM's collection are available at: <http://www.pharmanatur.com/Mycologie/Albomagister%20alesandrii.htm>.

Total DNA was extracted using the Wizard Genomic DNA kit (Proméga). The ITS1 - 5.8S - ITS2 region (ITS, Internal Transcribed Spacer) and part of the DNA coding for 28S ribosomal RNA was amplified by PCR (Polymerase Chain Reaction), using the primer pairs ITS1-F / ITS4B (Gardes & Bruns 1993) and LR0R / LR7 (Vilgalys & Hester 1990), respectively. Sequencing was performed by Eurofins Genomics (Ebersberg, Allemagne). Sequences were edited and corrected manually using BioEdit software (Hall 1999) and then aligned with Clustal W (Thompson et al. 1994). They were deposited in GenBank, under accession numbers MF344952 to MF344959 (Table 1).

The sequences of the different *Albomagister*, *Leucopaxillus*, *Porpoloma* and *Dennisiomyces* species generated by Sanchez-Garcia et al. (2014), *Tricholoma* sequences generated by Jargeat et al. (2010) and *A. alesandrii* sequences (Moreau et al. 2015) (Table 1) were included in the phylogenetic analysis performed with Mr Bayes v3.2 software (Ronquist et al. 2012), with six simultaneous Markov Chain Monte Carlo (MCMC) run over 2 000 000 generations, under GTR  $\beta$  gamma evolutionary model. Trees were saved every 500 generations, and the first 25 % were discarded as 'burn-in'. For the remaining trees, a majority rule consensus tree was computed to obtain estimates for Bayesian posterior probabilities. Phylogenetic trees were visualized and edited with FigTree v1.4.0, available at: <http://tree.bio.ed.ac.uk/software/figtree/>.

The alignment is available at <http://purl.org/phylo/treebase/phyloids/study/TB2:S21314>

**Table 1** Accession number of sequences used in this study

	ITS	28S
JMM01	MF344952	MF344956
JMM03	MF344953	MF344957
GC01	MF344954	MF344958
GC02	MF344955	MF344959
<i>Albomagister alesandrii</i>	KT315645	KT315644

**Table 1** Continued.

	ITS	28S
<i>Albomagister</i> sp. 2_MSG137	KJ417247	KJ417178
<i>Albomagister</i> sp.3_ECV4202	KJ417250	KJ417179
<i>Albomagister subaustralis</i> _ECV4049	KJ417251	KJ417180
<i>Albomagister subaustralis</i> _MGW676	KJ417252	KJ417181
<i>Dennisiomyces glabrescentipes</i> _JPF1000A	KJ417326	KJ417235
<i>Dennisiomyces</i> sp.1_DJL2103	KF291063	KF291064
<i>Dennisiomyces</i> sp.2_PR4763	KJ417269	KJ417191
<i>Dennisiomyces</i> sp.4_PR6334	KJ417271	KJ417192
<i>Dennisiomyces</i> sp.4_PR6613	KJ417268	KJ417190
<i>Dennisiomyces</i> sp.5_DLNC1505	KJ417327	KJ417239
<i>Dennisiomyces</i> sp.5_PBM3861	KJ417267	KJ417189
<i>Leucopaxillus albolactaceus</i> _LAS00.082	KJ417275	KJ417195
<i>Leucopaxillus amarus</i> _BW122201	KJ417278	KJ417197
<i>Leucopaxillus cerealis</i> _GB.0068845	KJ417282	KJ417198
<i>Leucopaxillus laterarius</i> _PBM3060	KJ417290	KJ417202
<i>Leucopaxillus lilacinus</i> _PBM3584	KJ417295	KJ417205
<i>Leucopaxillus tricolor</i> _TFB13462	KJ417323	KJ417207
<i>Porpoloma portentosum</i> _MES531	KJ417298	KJ417210
<i>Porpoloma portentosum</i> _REH5788	KJ417299	KJ417211
<i>Porpoloma sejunctum</i> _F0416	KJ417301	KJ417212
<i>Porpoloma</i> sp.1_PBM3238	KJ417304	KJ417214
<i>Porpoloma terreum</i> _F0030	KJ417306	KJ417216
<i>Porpoloma terreum</i> _REH5830	KJ417305	KJ417215
TENN064359_MBP060809	KJ417321	KJ417232
TENN064609_ECV4038	KJ417320	KJ417231
TENN068777_MSG144	KJ417322	KJ417233
<i>Tricholoma argyraceum</i> AF00.7	HQ184105	MF344961
<i>Tricholoma cingulatum</i> Rim03	GU060262	MF344963
<i>Tricholoma inocybeoides</i> Ville06	GU060266	MF344962
<i>Tricholoma scalpturatum</i> AF00.53	GU060279	MF344960

## Results

### *Phylogenetic analyses*

ITS and 28S sequences were obtained from samples GC01 and GC02, and from 2 specimens from JMM's collection (JMM01 and 03). The sequences of the 2 markers were combined to draw a unique phylogenetic tree (Fig. 1). The tree included *Albomagister*, *Leucopaxillus*, *Dennisiomyces*, *Tricholoma* and *Porpoloma* sequences, and was in perfect accordance with the one obtained by Sanchez-Garcia et al. (2014). Phylogenetic analysis showed that GC02, JMM01 and JMM03 sequences grouped with *Albomagister alesandrii* sequences (Fig. 1). Therefore they are now clearly identified as *A. alesandrii*.

Conversely, sequences from the GC01 specimen were closer to sequences from 2 American specimens (TENN064359 and TENN068777), for which no species name is currently available (Fig. 1). It therefore appears that, based on molecular analysis, GC01 should be attributed to a new species within *Albomagister*.

## Taxonomy

*Albomagister virgineus* Corriol & Jargeat, sp. nov.

Figs 2–5

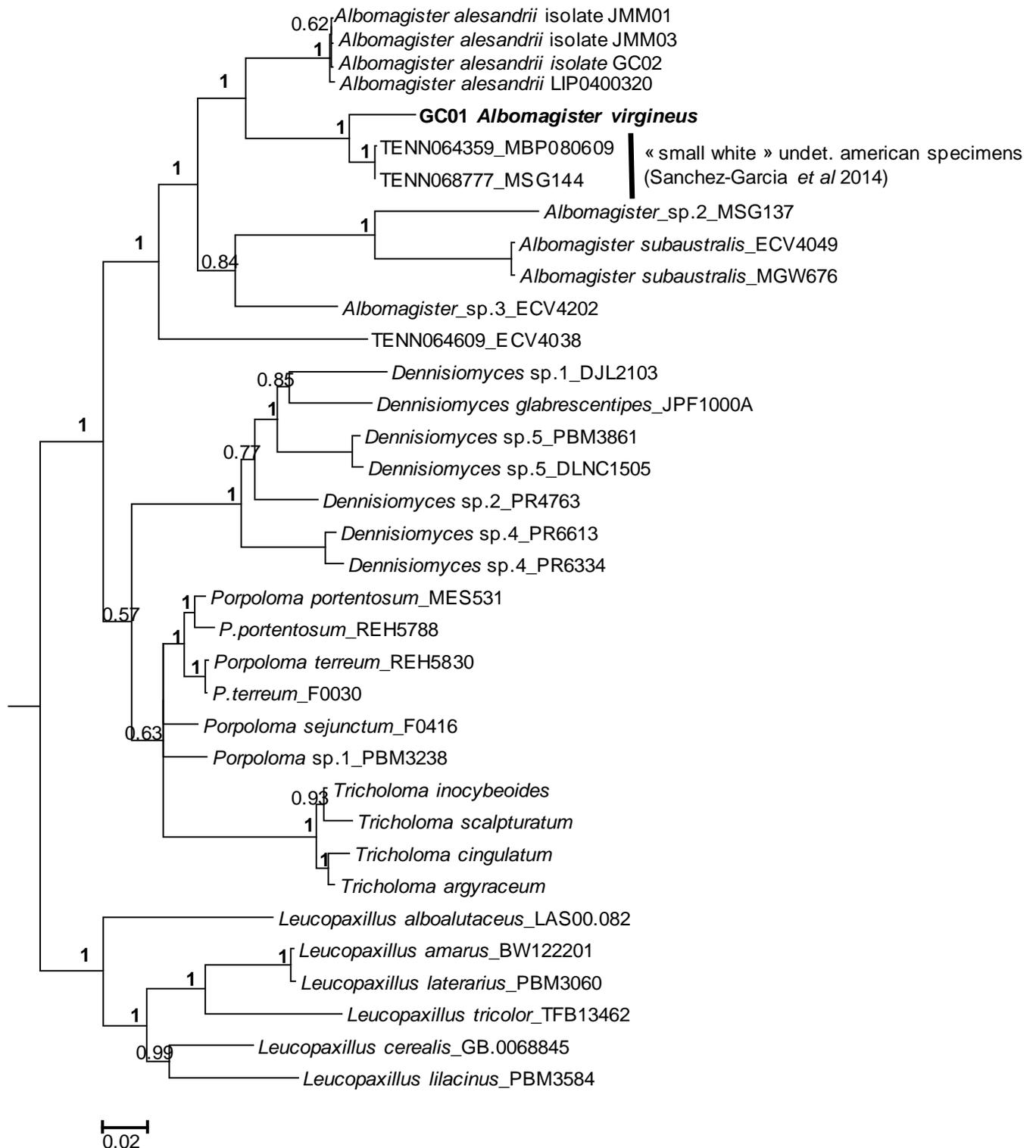
MycoBank number: MB821929

Etymology – from Latin *virginus*, virginal, in reference to its white colour.

Known distribution – up to now only known from *locus typicus*.

Material examined – France, Hautes-Pyrénées, Bagnères-de-Bigorre, on the hill that dominates the hamlet of Mespous, 821 m above sea level, in an old, dense boxwood (*Buxus*

*sempervirens* L.), shrub on calcareous bedrock, in thick litter, November 2007, G. Corriol, GC07110503 (BBF herbarium, **holotype**).



**Fig. 1** – Phylogenetic tree obtained from combined ITS and 28S sequences, drawn using MrBayes software program. Probabilities are indicated on each branch. GC01: collection GC07110503; GC02: collection GC01101108, JMM01 and JMM03: collection J.M. Moingeon (2011).

Diagnosis – Basidiome inocyboid, up to 11 mm diameter, conical-obtuse, white, fibrillose-silky, not hygrophanous. lamellae distant, emarginate, white turning yellowish with age. Stipe 15-

25 × 1-2 mm, undulate cylindrical, base clearly attenuate, slightly rooting; coating entirely flaky, pure white on a pure white background. Spores 5.8-6.7 × 4.9-6 μm, ellipsoid to subglobose, smooth, hyaline, wall more or less thickened, more than 0.5 μm thick in a few spores. Basidia 24-30 × 7-9 μm, 4-spored thin-walled, containing several oleiferous droplets. Cheilocystidia 8-52 × 5-10 μm, cylindrical, sometimes slightly globose or clavate. Sub-hymenium poorly developed, epicutis not much differentiated from subcutis, made of flat to slightly erect, entangled hyphae, deep pileal trama pseudoparenchymatous. Abundant clamp connections in all parts of the basidiome.

Differs from *Albomagister alesandrii* by the edge of its sterile lamellae, its cheilocystidia cylindrical or capitulate-apexed, its bigger spores and the frailer habitus of its basidiomata.

Pileus up to 11 mm, first lowly and obtusely conical and then almost plane, with a very slight umbo, a regular or irregular to lobate margin, a fibrillo-silky coating, very opaque, white, slightly ochraceous at the disc (2.5 Y 8/2), non hygrophorous, with a slightly exceeding, finely velvety, easily split margin; cap pale yellowish on exsiccata (2.5 Y 8/4 or a little paler).

Lamellae deeply emarginated, broad, 1.5 mm in width, spaced (25-35 large lamellae per basidiome), a few of them forked, usually 0-1 lamellula per lamella, nearly white (5 Y 8/1) when young, turning yellowish with age (5 Y 8/3), more deeply coloured, ochraceous on exsiccata (between 7.5 YR 6/8 and 10 YR 6/8), with a white edge, densely pubescent when observed under the binocular magnifier.

Stipe 15-25 × 1-2 mm, undulate cylindrical, base clearly attenuate, slightly radicate; coating entirely flaky, pure white on a pure white background; full.

Flesh thin, from 0.3 mm thick at mid-radius to 1 mm in umbo, smell not perceivable, taste distinctly bitter and slightly astringent.

Spore print white.

Spores D1, 9 [N=32] (5,6)5,8-6,2-6,7(7,6) × (4,6)4,9-5,3-6(6,15) μm; Q = (1,04)1,08-1,17-1,27(1,34), largely ellipsoid to sub-globose, often with a slightly wider base, apex 0.5-1 μm, smooth, hyaline, wall more or less thickened, more than 0.5 μm thick in a few spores, wall cyanophilous, all the more so as it is thick (strongly cyanophilous in thick-walled spores), these few thick-walled spores also congophilous, with a dented-subangular appearance, with a living content generally presenting a large oleiferous droplet dispersed into numerous small clusters in lethal reagents, partially metachromatic; their reaction to iodine is variable: it goes unnoticed on most spores (vaguely greyish ?) whereas thick-walled spores are more or less strongly dextrinoid (red-brown), and were only observed on hymenium covered with spores from mature basidiomata (not on the sparse spore print); they only represented a few percent of all spores.

Basidia 24-30 × 7-9 μm, clavate, tetrasporic, with clamp connections, non-reactive to iodine, thin-walled (no sclerobasidia observed), containing several oleiferous droplets.

Cheilocystidia 8-52 × 5-10 μm, cylindrical, sometimes slightly ventricose or clavate, apex frequently capitulate, some of them articulate, densely covering the sterile edge; the edge observed in cresyl blue showed metachromatic exudates unobserved with other reagents.

Absence of pleurocystidia.

Lamella trama parallel, hyphae with ventricose articles, 4-10(15) μm in width.

Sub-hymenium poorly developed, shortly articulate.

Epicutis not much differentiated from subcutis, made of flat to slightly erect, entangled hyphae, with long articles up to 200 μm long and 5-15 μm wide, with a few ends erect and more shortly articulate.

Deep pileal trama pseudoparenchymatous, with short, 15-25 μm-wide articles.

Stipe covered with numerous tufts of articulate, clavate-ended hairs.

Pigment hard to detect; we hypothesize that it is yellowish, smooth parietal.

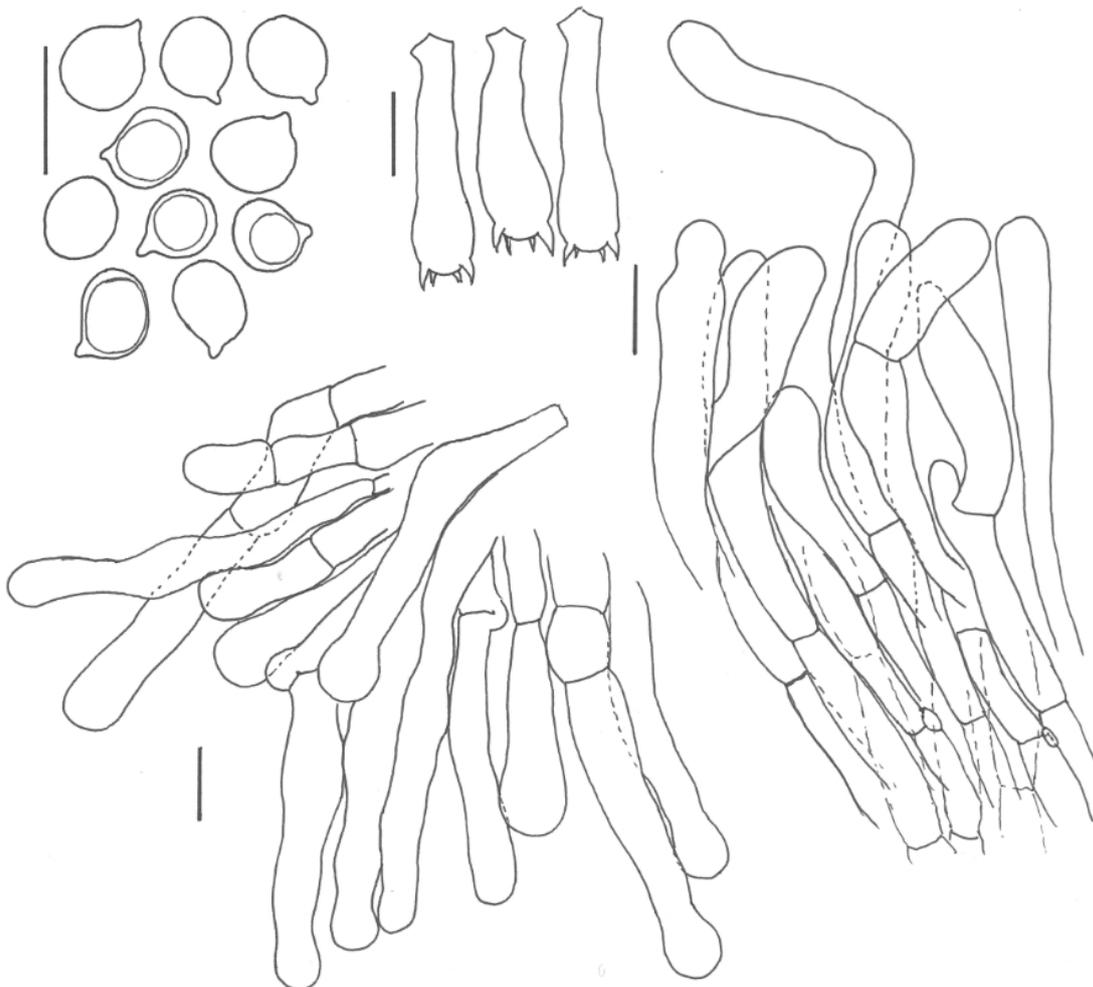
Abundant clamp-connections in all parts of the basidiome.

## Discussion

The circumscription of the *Albomagister* genus is currently still rather unclear. However, we must admit that we had not contemplated classifying our collection in *Tricholoma*, the closest European genus known to date (Sanchez-Garcia et al. 2014). The characters given for the *Albomagister* genus, typified by a species initially described in the *Hygrophorus* genus (*H. subaustralis* A.H. Smith & Hesler), are the following: “habit tricholomatoid; lamellae sinuate to adnexed, white, edges concolorous. Spores inamyloid, smooth, thin-walled. Cheilo- and pleurocystidia present and conspicuous, thin-walled and hyaline. Pileipellis with ascending interwoven cylindrical elements. Clamp connections present. On soil, in forests beneath woody vascular angiosperms and coniferous plants. Probably ectomycorrhizal.”

Differences with the *Tricholoma* are thought to be related to the presence of noticeable pleuro- and cheilocystidia of a deeper origin, whereas they are not easily noticed and of hymenial origin in the *Tricholoma* genus (Sanchez-Garcia et al. 2014).

Only three *Albomagister* species have been described to date (*A. subaustralis*, *A. alesandrii* and *A. virgineus*), but in their cladogram Sánchez-García et al. (2014) mentioned two other species genetically affiliated to the *Albomagister* genus (noted sp. 2 and sp. 3) whose circumscription is currently unknown. Besides, three other unidentified collections are in basal position on two distinct branches of their “*Albomagister*” clade. Two of them (TENN064359 and TENN068777) are genetically closest to *A. virgineus*. Sánchez-García et al. (2014, page 1003) also indicate that *A. subaustralis sensu* Dennis (1953), collected in Cuba, and could correspond to another species.



**Fig. 2** – *Albomagister virgineus*, holotype: top left, spores; top, basidia; bottom left, part of the edge of a lamella; bottom right, length-cut of stipe cuticle; scale bars: 10  $\mu$ m; drawings by GC.

The GC02 collection, identified as *A. alesandrii*, allowed us to further detail the variability of certain characters not evidenced by the sole basidiome from an unknown habitat used for the original description of *Albomagister alesandrii* (Moreau et al. 2015), in particular the strong dextrinoidy of certain thick-walled spores (a low percentage in the order of 1 or a few %), the shape of cheilocystidia, frequently with a long, narrow, flexuose neck, the frail habitus of basidiomes. Smaller spores (D1, 9 [N=24] (4,1)4,3-4,8-5,2(5,3) × (3,3)3,5-4,0-4,3(4,7) μm; Q = (1,05)1,08-1,21-1,32(1,40) ) have also been observed.

The description of the two monophyletic European species within the *Albomagister* genus (*A. alesandrii* and *A. virgineus*) questions the definition of the genus proposed in its original description (Sánchez-García et al. 2014). The habitus of *A. alesandrii* is rather frail-inocyboid than tricholomatoid, whereas *A. virgineus* is even frailer and has an inocyboid to mycenoid habitus (Table 2). Moreover, *A. virgineus* is deprived of pleurocystidia, whereas *A. alesandrii* pleurocystidia are restricted to the immediate vicinity of the edge. Finally, the spores of the two species are reactive to iodine and thick-walled. Both species were found in dense boxwood shrub, so their mycorrhizal character is questionable, as *Buxus sempervirens* L. is not known to develop ectomycorrhizae. The only ectomycorrhizal plant likely to be found close enough to our stations could be hazelnut tree (*Corylus avellana* L.) or possibly shrubby Rosaceae (*Crataegus* sp.). Therefore the only reliable characters left in the original description are the white, emarginated (sinuate) to adnexed lamellae, the smooth spores, the presence of hyaline, thin-walled, clearly differentiated cheilocystidia, the entangled ascending epicutis, and the presence of clamps. The remaining differential characters with the *Tricholoma* genus are the presence of strongly differentiated, deeply rooted cheilocystidia.

These differences find an echo from a molecular point of view. Although the two European species are monophyletic with *A. subaustralis*, the genus holotype, they are both born by the same diverging branch of the dendrogram. These differences lead us to wonder whether it is relevant to make a distinction at a supraspecific taxonomic level allowing for a better circumscription of each clade. Maybe the discovery of new species belonging to the *Albomagister* genus, and more numerous, detailed macro- and micro-anatomical studies, especially concerning the *exsiccata* in which non-identified sequences were found (TENN064359 et TENN068777) introduced by Sanchez-Garcia et al. (2014) in the phylogenetic tree of the *Albomagister* genus will lead to a better circumscription of the genus.

**Table 2** Comparison of a few characters of three currently described *Albomagister* species

	<i>A. subaustralis</i> *	<i>A. alesandrii</i>	<i>A. virgineus</i>
Habitus	Tricholomatoid	Inocyboid	Very frail inocyboid, nearly mycenoid
Spores, reaction to iodine	None	A few dextrinoid spores	A few dextrinoid spores
Spores wall	Thin	Some thick-walled spores	Some thick-walled spores
Lamella edge	Heterogeneous	Heterogeneous	Sterile
Pleurocystidia	Present	Present near the edge	Absent
Cystidia	globose-fusoïd	globose-fusoïd	Cylindrical to slightly capitulate
Nutrient intake	Ectomycorrhizal	Saprotrophic ?	Saprotrophic ?

\* According to Smith & Hesler (1942)

**Determination key of the species currently known in the *Albomagister* genus<sup>1</sup>**

1. Presence of dextrinoid sclerobasida; most spores dextrinoid, ≤ 6 x 4.5 μm ..... *Pseudobaeospora* genus
- 1'. Absence of sclerobasida; at most a low proportion of larger dextrinoïdes spores..... 2
2. Tricholomatoid habitus, thin-walled spores; pleurocystidia present on the two faces of the lamellae. North America.....  
.....*A. subaustralis* (A.H. Smith & Hesler) Sánchez-García, Birkebak & Matheny<sup>2</sup>

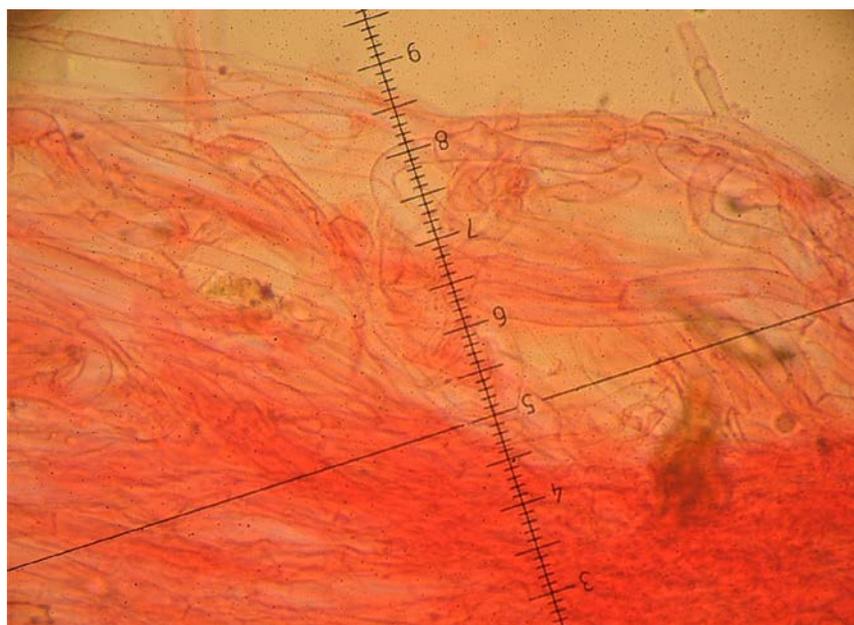
- 2'. Frail inocyboid to mycenoid habitus, at least a few thick-walled spores; pleurocystidia present near the edge at most. Europe..... 3
3. Edge of lamellae sterile, densely covered with cylindrical to capitulate cystidia; spores  $> 6 \times 5 \mu\text{m}$  on average; pleurocystidia absent ..... *A. virgineus* Corriol & Jargeat
- 3'. Edge of lamellae heterogeneous, with attenuated cystidia, often with a long flexuose neck; spores  $< 6 \times 5 \mu\text{m}$  on average; pleurocystidia similar to cheilocystidia near the edge .....  
 ..... *A. alesandrii* P.-A. Moreau, Bellanger, Biancardini & F. Richard

<sup>1</sup> Sánchez-García et al. (2014) mention other species genetically affiliated to the *Albomagister* genus, but whose circumscription is currently unknown.

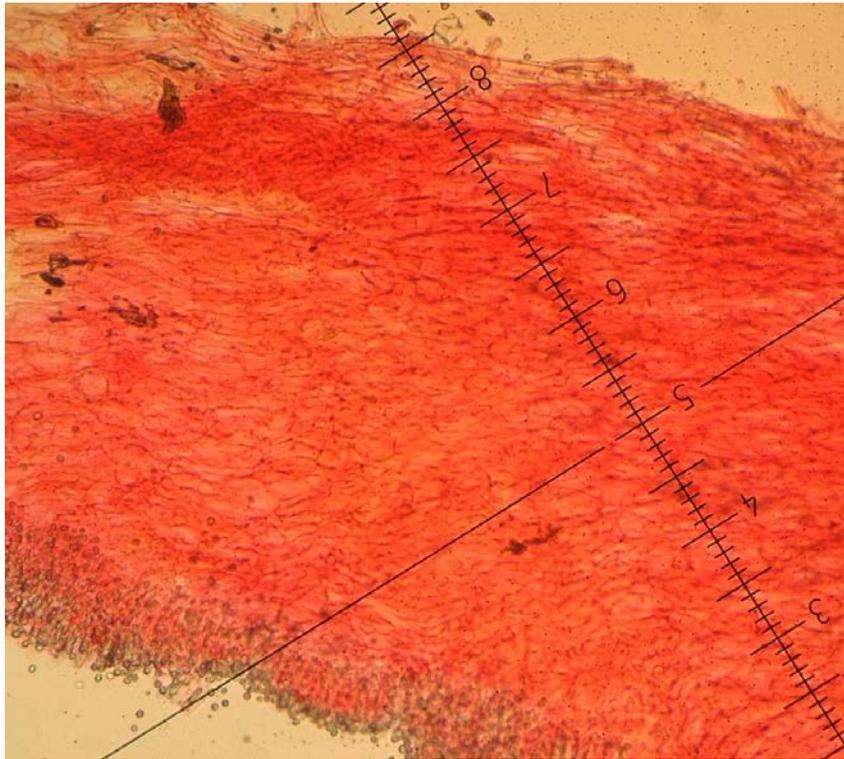
<sup>2</sup> With attenuate cystidia, see *Hygrophorus subaustralis sensu* Dennis (1953) from Cuba.



**Fig. 3** – *Albomagister virgineus*, holotype *in situ*, in the humus of a dense boxwood shrub.



**Fig. 4** – *Albomagister virgineus*, epicutis in congo red; graduations = 2.5  $\mu\text{m}$ .



**Fig. 5** – *Albomagister virgineus*, pileus trama in congo red; graduations = 10 µm.



**Fig. 6** – *Albomagister alesandrii*, collection of Oct. 11<sup>th</sup>, 2001, in a boxwood shrub at Courcelles-les-Quingey (Doubs, France), (herb. GC01101108).

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