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Typification of the four most investigated and valuable truffles:  
*Tuber aestivum* Vittad., *T. borchii* Vittad.,  
*T. magnatum* Picco and *T. melanosporum* Vittad.

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# Typification of the four most investigated and valuable truffles: *Tuber aestivum* Vittad., *T. borchii* Vittad., *T. magnatum* Picco and *T. melanosporum* Vittad.

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## ABSTRACT

The true truffles, *Tuber aestivum* Vittad., *T. borchii* Vittad., *T. magnatum* Picco and *T. melanosporum* Vittad., are among the most studied fungal species; they also have a high economic value due to their special aromatic and nutritional properties that make them a much sought delicacy. Despite this, their identification has been based on morphological and then molecular characters in the absence of reference type specimens. Although long of scientific, commercial and regulatory use, these four scientific names are at risk due to a lack of nomenclatural priority. To provide the scientific community with reference voucher samples and to initiate nomenclatural proposals for the recognition of their status as conserved names, three collections from sites mentioned by their authors (Picco and Vittadini) are proposed as epitypes for *Tuber aestivum*, *T. borchii* and *T. melanosporum*, and one as a neotype for *T. magnatum*. The type of each name is described morphologically and molecularly characterized with the sequences of three markers: ITS,  $\beta$ -tubulin, elongation factor 1 $\alpha$ . The taxonomy and nomenclature of each species are discussed. The conservation of the names *Tuber aestivum* against the previous homonymous *Tuber aestivum* (Wulfen) Spreng. and the competing name *Tuber blotii* Eudes-Desl., *T. magnatum* against *Tuber griseum* Borch ex Pers., and *T. melanosporum* against *Tuber nigrum* Bull. will be proposed. The name *Tuber borchii* has no previous synonyms and therefore it is legitimate and does not require conservation.

## KEY WORDS

Multilocus typing,  
nomenclature,  
ITS,  
 $\beta$ -tubulin,  
elongation factor 1 $\alpha$ ,  
history,  
lectotypifications,  
néotypifications,  
épitypifications.

## RÉSUMÉ

*Typification des quatre truffes les plus recherchées et les plus précieuses* : *Tuber aestivum* Vittad., *T. borchii* Vittad., *T. magnatum* Picco et *T. melanosporum* Vittad.

Les vraies truffes *Tuber aestivum* Vittad., *T. borchii* Vittad., *T. magnatum* Picco et *T. melanosporum* Vittad. sont parmi les espèces de champignons les plus étudiées ; en outre elles ont une valeur économique élevée, due à leurs caractéristiques aromatiques et nutritives qui en font une gourmandise gastronomique. Malgré cela, leur identification s'est toujours basée sur l'analyse des caractères morphologiques et moléculaires en absence de spécimen type de référence. Bien que les noms aient été largement utilisés à des fins aussi bien scientifiques que commerciales, l'appellation scientifique est confrontée dès le départ au risque lié à la priorité nomenclaturale et sa sanction légale. Afin de fournir à la communauté scientifique des échantillons de référence et d'avancer la proposition nomenclaturale pour la reconnaissance de leur statut de « nomina conservanda », quatre collections provenant des lieux indiqués par leurs auteurs (Picco et Vittadini) sont proposées comme épitypes pour *Tuber aestivum*, *T. borchii* and *T. melanosporum*, et néotypes pour *T. magnatum*. Le type de chaque nom est décrit aussi bien morphologiquement que moléculairement par les séquences de trois marqueurs ITS,  $\beta$ -tubuline et le facteur d'élongation 1 $\alpha$ . La taxonomie et la nomenclature de chaque espèce sont discutées. La conservation des noms *Tuber aestivum* contre l'homonyme *Tuber aestivum* (Wulfen) Spreng. et le synonyme précédent *Tuber blotii* Eudes-Desl., *T. magnatum* contre *Tuber griseum* Borch ex Pers. et *T. melanosporum* contre *Tuber nigrum* Bull. sera proposé. Le nom *Tuber borchii* n'a pas de synonyme précédent, il est donc légitime et ne nécessite pas de conservation.

## MOTS CLÉS

Multilocus typing, nomenclature, ITS,  $\beta$ -tubuline, facteur d'élongation 1 $\alpha$ , histoire, lectotypifications, neotypifications, epitypifications.

## INTRODUCTION

Truffles in the genus *Tuber* P. Micheli ex F.H. Wigg. (Ascomycota, Pezizales) are edible subterranean fruiting bodies (ascomata) regarded since ancient times as a luxury food icon. They live in symbiosis with the root systems of trees and shrubs. *Tuber* species are native to the northern hemisphere (Bonito *et al.* 2013). However, the possibility to cultivate some species associated with suitable host plants has allowed their introduction into the southern hemisphere. The most important species cultivated worldwide are *Tuber aestivum* Vittad. (the summer truffle), *T. borchii* Vittad. (bianchetto) and *T. melanosporum* Vittad. (the Périgord truffle). Only the Italian white truffle (*Tuber magnatum* Picco) has yet to be successfully domesticated and production is concentrated within natural areas of the Italian, Balkan and Anatolian peninsulas (Belfiori *et al.* 2020; unpublished data). The geographic distribution of these species is closely associated with their genetic variability; *T. magnatum* being the least variable species and hence with the most restricted occurrence (Mello *et al.* 2006, 2017).

With the advent of molecular biology and the “-omics” era, these studies on population genetics, ecological and functional interactions and VOCs production significantly increased (Pacioni *et al.* 2015; Zambonelli *et al.* 2016; Mello *et al.* 2017). By 2018, the sequenced genomes within the genus *Tuber* included *T. aestivum* and *T. magnatum* (Murat *et al.* 2018a), *T. borchii* (Murat *et al.* 2018b) and *T. melanosporum* (Martin *et al.* 2010).

The scientific and economic interest regarding these truffle species is hampered by lack of taxonomic and nomenclatural references because no type specimens were designated by the authors of the four species. Images of *T. aestivum*, *T. borchii* and *T. melanosporum* can serve as lectotypes, which in the

case of *Tuber aestivum* are also accompanied by figures of the ascospores in the asci (Vittadini 1831). *Tuber magnatum* is devoid of illustrations or specimens by Picco (1788) so it is necessary to propose a neotype. These four names are used in the laws and regulations of many states and also in international trade agreements, however they may or may not comply with the rules of the nomenclature code (International Code of Botanical Nomenclature 1983; Ceruti *et al.* 2003; Turland *et al.* 2018). The distinctive characteristics of these truffle species have been those identified by their morphology and then consolidated by DNA sequencing without ever having had any reference types to ensure accurate future identification.

For these reasons it is necessary to designate epitype specimens of *Tuber aestivum*, *T. borchii*, and *T. melanosporum* and a neotype of *T. magnatum* based on recent collections from the areas indicated by their authors in the protologues for the names (Picco 1788; Vittadini 1831).

## MATERIAL AND METHODS

### SPECIMENS

No authentic herbarium samples of *Tuber magnatum* Picco (1788) exist, while authentic material from Vittadini (1831) of *T. aestivum*, *T. borchii* and *T. melanosporum* in herbaria Kew (K), Padova (PAD), Torino (TO) and Uppsala (UPS) lack adequate collecting data to determine their status as original materials. Vittadini's specimens from herbaria were used only as morphological reference material, because the ascomatal fragments are small, sometimes highly compromised by use of chemicals applied for preservation, or moulds or completely immature as for *T. aestivum* at UPS. The type vouchers are preserved at the University Herbaria of Torino and L'Aquila (AQU).

## COLOR

Color names of ascomata were selected according to the HEXA ([htmcolorcodes.com/color-picker](http://htmcolorcodes.com/color-picker), [encycolorpedia.com](http://encycolorpedia.com)).

## MICROSCOPY

Microscopy observations, photos and measurements were conducted with a Leica DFC 450 digital camera coupled with a Leica DM 2500 microscope. Herbarium specimens were treated according to Leonardi *et al.* (2019) by rehydrating the samples with 20% KOH and observing in 3% KOH, after prolonged capillary washing with distilled water. To study peridium structure, small portions of ascomata were fixed overnight at 4°C with glutaraldehyde 2.5% in PBS (phosphate saline buffer) 10 mM pH 7.2 solution. Cryosections 10 µm thick were prepared with a cryostat (2800 Frigocut E, Reichert Jung, Germany) and TissueTec OCT-compound (Miles, Elkhart, Ind., United States). The sections were transferred onto glass slides and air-dried for 1 h. A small drop of Amann's solution (phenol, 20 g; lactic acid, 16.5 mL; glycerol, 32 mL; distilled water, 20 mL) was used as mounting medium. Double measurements joined by a dash indicate the minimum and maximum values found.

## DNA EXTRACTION, AMPLIFICATION AND SEQUENCING

Genomic DNAs were isolated from 20 mg of each dried sample by the DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions and were eluted in 70 µL of sterile water. The DNA quality and concentration of each sample were evaluated on 1% agarose gel and estimated with a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Madison, WI). Internal transcribed spacer regions (ITS), the β-tubulin (β-tub) and elongation factor 1-α (EF) genes were amplified according to procedures described in Glass & Donaldson (1995), Leonardi *et al.* (2005) and Paolocci *et al.* (2004) respectively with primers pair ITS1F/ITS4 for the ITS regions (White *et al.* 1990; Gardes & Bruns 1993), Bt2a/Bt2b for the β-tub gene and EFtFw/ EFtBw for the EF gene (Paolocci *et al.* 2004). Amplification reactions were performed in a SimpliAmp™ Thermal Cycler (Life Technologies). Amplicons were purified with the QIAquick PCR Purification Kit (Qiagen, Milan, Italy) following the manufacturer's instructions and sequenced by Eurofins Genomics service (Ebersberg, Germany). The new sequences were submitted to GenBank and their accession numbers are reported in type descriptions below.

## ITS SEQUENCE ANALYSIS

Phylogenetic analysis was carried out on ITS sequences generated from *T. aestivum*, *T. borchii*, *T. melanosporum* epitypes and *T. magnatum* neotype and those of other *Tuber* species retrieved from GenBank (Table 1). The Maximum Likelihood (ML) tree was inferred with raxmlGUI 1.5b2 (Silvestro & Michalak 2012). An ITS sequence of *Pithya vulgaris* (GenBank accession number U66008; Roux *et al.* 1999) was used as outgroup. Multiple sequence alignment was performed in MAFFT 7 (Katoh & Standley 2013) with the E-INS-i iterative refinement algorithm. ML analysis was performed with

100 rapid bootstrap replicates (10 runs) and application of the GTRGAMMAI model of nucleotide substitution. The analysis involved 109 nucleotide sequences having 952 positions in the final dataset.

## RESULTS

Morphological and anatomical features of the specimens proposed as epitypes and neotype agree with those provided by researchers and stakeholders, traditional knowledge, plus the descriptions of Vittadini (1831) and morphology of his herbarium samples. The succinct description of *T. magnatum* by Picco (1788) also coincides with the present general agreement, inasmuch as it is a species known and marketed for centuries in northeastern Italy.

Molecular analyses unequivocally confirm their identity according to current phylogenetic knowledge of the *Tuber* genus (Fig. 1).

Family TUBERACEAE Dumort.  
Genus *Tuber* P. Micheli ex F.H. Wigg.

*Tuber aestivum* Vittad.  
(Figs 3; 4A; 5A, B; Appendix 1)

*Monographia Tuberacearum*: 38 (1831).

MYCOBANK. — MB 218597.

GENBANK. — MZ423173 (nrITS), MZ458417 (nr β-tubulin), MZ458421 (nrEF 1-α).

LECTOTYPE OF *TUBER AESTIVUM* VITTAD. — Vittadini 1831: tab. II fig. IV C, D (here designated; MycoBank Typification number: MBT 10001890; Fig. 3).

EPITYPE *TUBER AESTIVUM* VITTAD. — Italy. Lombardy, Monza, Parco Villa Reale, sub *Tilia cordata* Mill., 8.IX.2019, 45°37'09"N, 9°16'48"E, five ascomata, leg. Stefano Seghezzi, det. Giovanni Pacioni (epi-, AQUI[AQUI 10150], here designated; MycoBank Typification number: MBT 10001891).

Authentic Vittadini specimens examined: K(M) 254890, TO and UPS (F-628206).

## DESCRIPTION

*Ascomata*

Hypogeous, globose or irregular, up to 10 cm in diameter or more, rarely with a flat or slightly basal excavation, surface of peridium black to very dark gray (#000000 to 333333) in both mature and immature specimen, covered with pyramidal warts polygonal at the base, with 4-7 sides, 3-13 mm wide × 0.5-5.0 mm high, generally acute but often depressed or concave, with sharp edges or with longitudinal radial fissures, surface of the warts typically with evident parallel transverse streaks.

*Gleba*

Firm and compact, whitish (FAE5D3 light greyish orange) to more or less brown (E59866 soft orange, 6E2C00, very dark

TABLE 1. — Information on specimens and DNA sequences analyzed in this study. (\* To comply with ICN - Shenzhen - Art. 60.10, this must be corrected to: *Tuber rapiodorum*).

Taxon	ITS GenBank accession #	Sample name	Country	References
<i>Tuber aestivum</i> Vittad.	MZ423173	AQUI 10150	Italy: Lombardy, Monza, Parco Villa Reale	this study
	AF516779	strain E32	Italy: Molise	Paolocci <i>et al.</i> 2004
	AF516781	strain E2	Italy: Abruzzo	Paolocci <i>et al.</i> 2004
	AF516782	strain Ep8	Italy: Piemonte	Paolocci <i>et al.</i> 2004
	AF516783	strain E61	Italy: Umbria	Paolocci <i>et al.</i> 2004
	AF516784	strain Ep7	Italy: Piemonte	Paolocci <i>et al.</i> 2004
	AF516785	strain E50	Italy: Umbria	Paolocci <i>et al.</i> 2004
	AF516786	strain E58	Italy: Umbria	Paolocci <i>et al.</i> 2004
	AF516787	strain E29	Italy: Abruzzo	Paolocci <i>et al.</i> 2004
	AF516788	strain E1	Italy: Abruzzo	Paolocci <i>et al.</i> 2004
	AF516789	strain E60	Italy: Umbria	Paolocci <i>et al.</i> 2004
	AF516790	strain E18	Italy: Abruzzo	Paolocci <i>et al.</i> 2004
	AF516791	strain E5	Italy: Molise	Paolocci <i>et al.</i> 2004
	AF516792	strain E24	Italy: Emilia Romagna	Paolocci <i>et al.</i> 2004
	AY226042	strain E17	Italy: Abruzzo	Paolocci <i>et al.</i> 2004
	AF132509	<i>Tuber uncinatum</i>	unknown	Roux <i>et al.</i> 1999
	<i>Tuber borchii</i> Vittad.	MZ423174	AQUI 10151	Italy: Lombardy, Varzi, Castello Oramala
DQ679802		isolate 17Bo	Italy: Emilia Romagna, Ravenna—Marina di Ravenna	Bonuso <i>et al.</i> 2010
FJ554490		voucher CMI-UNIBO 2445	Italy: Lombardia, Pavia	Bonuso <i>et al.</i> 2010
FJ554506		voucher CMI-UNIBO 3058	Italy: Emilia Romagna, Ferrara—Gardelletta	Bonuso <i>et al.</i> 2010
FJ554507		voucher CMI-UNIBO 3087	Italy: Emilia Romagna, Ferrara—Vascello d'Oro	Bonuso <i>et al.</i> 2010
FJ554466		voucher CMI-UNIBO Tbo1570	Italy: Emilia Romagna, Bologna—Sasso Marconi	Bonuso <i>et al.</i> 2010
FJ554485		voucher CMI-UNIBO 1833	Italy: Emilia Romagna, Reggio Emilia—Quattrocastella	Bonuso <i>et al.</i> 2010
FJ554470		voucher CMI-UNIBO Tbo2352	Italy: Veneto, Rovigo—Porto Viro	Bonuso <i>et al.</i> 2010
FJ554469		voucher CMI-UNIBO Tbo2364	Italy: Veneto, Rovigo—town cemetery	Bonuso <i>et al.</i> 2010
FJ554481		voucher CMI-UNIBO 2387	Italy: Molise, Isernia—Belmonte del Sannio	Bonuso <i>et al.</i> 2010
FJ554513		voucher CMI-UNIBO 3300	Italy: Sicilia, Palermo—Monte Petroso	Bonuso <i>et al.</i> 2010
FJ554493		voucher CMI-UNIBO 3026	Italy: Emilia Romagna, Ferrara—Bosco Spada	Bonuso <i>et al.</i> 2010
FJ554502		voucher CMI-UNIBO 3065	Italy: Emilia Romagna, Ferrara—Gran Bosco della Mesola	Bonuso <i>et al.</i> 2010
FJ554495		voucher CMI-UNIBO 3078	Italy: Sicilia, Palermo—San Martino delle Scale	Bonuso <i>et al.</i> 2010
FJ554491		voucher CMI-UNIBO 2363	Italy: Veneto, Rovigo—town park	Bonuso <i>et al.</i> 2010
AJ002510		isolate 48 1992	unknown	Mello <i>et al.</i> 1998
<i>Tuber magnatum</i> Picco		MZ423175	TO HG3458	Italy: Piedmont, Montechiaro D'Asti, Loc. Seria
	AJ586268	isolate B1	Italy: Piedmont, San Desiderio (AT)	Mello <i>et al.</i> 2002
	AJ586267	isolate B2	Italy: Piedmont, Zanco (AT)	Mello <i>et al.</i> 2002
	AJ586266	isolate B3	Italy: Piedmont, Ponzano (AC)	Mello <i>et al.</i> 2002
	AJ586271	isolate B6	Italy: Piedmont, San Desiderio (AT)	Mello <i>et al.</i> 2002
	AJ586265	isolate B8	Italy: Piedmont, Castellalfero (AT)	Mello <i>et al.</i> 2002
	AJ586264	isolate B9	Italy: Piedmont, Castellalfero (AT)	Mello <i>et al.</i> 2002
	AJ586263	isolate B19	Italy: Piedmont, Montemagno (AT)	Mello <i>et al.</i> 2002
	AJ586262	isolate B11	Italy: Piedmont, Montemagno (AT)	Mello <i>et al.</i> 2002
	AJ586261	isolate E1	Italy: Piedmont, Montemagno (AT)	Mello <i>et al.</i> 2002
	AJ586260	isolate b2	Italy	Mello <i>et al.</i> 2005
	AJ586259	isolate b4	Italy	Mello <i>et al.</i> 2005
	AJ605110	isolate Tmsal	Italy: Campania, Salerno	Mello <i>et al.</i> 2005
	AJ586258	isolate xl	Italy: Molise, Campobasso	Mello <i>et al.</i> 2005
	AJ586257	isolate y	Italy: Molise, Campobasso	Mello <i>et al.</i> 2005
	AJ586256	isolate z	Italy: Molise, Campobasso	Mello <i>et al.</i> 2005
	AJ002509	isolate 111992	unknown	Mello <i>et al.</i> 1998
<i>Tuber melanosporum</i> Vittad.	MZ423176	AQUI 10152	Italy: Lombardy, Monza, Parco Villa Reale	this study
	EU200420	isolate 84	Spain: Sierra de Loquiz - Navarra	Riccioni <i>et al.</i> 2008
	EU200421	isolate 281	Italy: Spoleto Loc. S.Maria di Reggiano - Perugia	Riccioni <i>et al.</i> 2008
	EU200422	isolate 121	France: Provence	Riccioni <i>et al.</i> 2008
	EU200433	isolate MEL178	France: Provence - Tavernes	Riccioni <i>et al.</i> 2008
	EU200434	isolate MEL199	Italy: Cerreto di Spoleto - Perugia	Riccioni <i>et al.</i> 2008
	EU200410	isolate MEL298	France: Borgogne - Yonne	Riccioni <i>et al.</i> 2008

TABLE 1. — Continuation.

Taxon	ITS GenBank accession #	Sample name	Country	References
	EU555383	isolate MEL178 clone 2	France: Provence - Tavernes	Riccioni <i>et al.</i> 2008
	EU555384	isolate MEL178 clone 10	France: Provence - Tavernes	Riccioni <i>et al.</i> 2008
	EU555385	isolate MEL178 clone 49A	France: Provence - Tavernes	Riccioni <i>et al.</i> 2008
	EU555386	isolate MEL199 clone 11	Italy: Cerreto di Spoleto - Perugia	Riccioni <i>et al.</i> 2008
	EU555387	isolate MEL298 clone 23	France: Borgogne - Yonne	Riccioni <i>et al.</i> 2008
	EU555388	isolate MEL437 clone 13A	Italy: Spoleto - Perugia	Riccioni <i>et al.</i> 2008
	EU555389	isolate MEL437 clone 25A	Italy: Spoleto - Perugia	Riccioni <i>et al.</i> 2008
	EU555390	isolate MEL437 clone 26A	Italy: Spoleto - Perugia	Riccioni <i>et al.</i> 2008
	EU555391	isolate MEL437 clone 30A	Italy: Spoleto - Perugia	Riccioni <i>et al.</i> 2008
	EU555392	isolate MEL282 clone 48A	Italy: Spoleto Loc. S.Maria di Reggiano - Perugia	Riccioni <i>et al.</i> 2008
	AF132501	<i>Tuber melanosporum</i>	unknown	Roux <i>et al.</i> 1999
<i>Tuber brumale</i> Vittad. var. <i>moschatum</i> (Bull.) I.R. Hall, P.K. Buchanan, Wang & Cole	AF001010	<i>Tuber brumale</i> f. <i>moschatum</i>	central Italy	Rubini <i>et al.</i> 1998
<i>Tuber brumale</i> Vittad.	AF106880	clone B44	Italy: central, Marche	Unpublished
	JF926118	isolate S72	Germany: Baden-Württemberg	Stobbe <i>et al.</i> 2012
	AF132504	<i>Tuber brumale</i>	unknown	Roux <i>et al.</i> 1999
<i>Tuber excavatum</i> Vittad.	HM152011	isolate Tub09	Austria: Bezirk Klosterneuburg, Kritzensdorf	Unpublished
	JF926119	isolate S63	Germany: Baden-Württemberg	Stobbe <i>et al.</i> 2012
	HM151997	isolate it7_0	Germany: Zierenberg	Unpublished
	FN433146	specimen voucher SFI:TUBEXC/250109B	Slovenia: Nanos	Unpublished
<i>Tuber excavatum</i> Vittad. var. <i>intermedium</i> G. Gross	KX354287	voucher JS812014	Germany: north-east Bavaria, Nördliche Frankenalb	Schiebold <i>et al.</i> 2017
<i>Tuber macrosporum</i> Vittad.	AF106885	clone Macro1	Italy: central, Umbria	Unpublished
	KP738396	voucher ITA_011s	Italy	Benucci <i>et al.</i> 2016
	KP738349	voucher ITA_013s	Italy	Benucci <i>et al.</i> 2016
	KP738346	voucher ITA_012S	Italy	Benucci <i>et al.</i> 2016
	KP738351	voucher ITA_014s	Italy	Benucci <i>et al.</i> 2016
<i>Tuber mesentericum</i> Vittad.	AF132508	<i>Tuber mesentericum</i>	unknown	Roux <i>et al.</i> 1999
	JF926122	isolate S7509	Germany: Baden-Württemberg	Stobbe <i>et al.</i> 2012
	AF516799	strain Mg	unknown	Unpublished
	FM205537	isolate 04MES	Slovenia: Karst region	Unpublished
	HM485375	isolate CW105	Sweden: Gotland	Bonito <i>et al.</i> 2010
<i>Tuber maculatum</i> Vittad.	AF106889	clone Mac1	Italy: central, Umbria	Unpublished
	AJ879691	isolate 2	Italy: Piedmont	Murat <i>et al.</i> 2005
	EU753269	herbarium 1967	Italy	Unpublished
	AJ969627	specimen voucher Vittad. TL5974 (Copenhagen Botanical Museum)	Denmark: Gurre Sø, North East Zealand	Tedersoo <i>et al.</i> 2006
<i>Tuber dryophilum</i> Tul.	JQ925644	voucher GB35	Italy	Bonito <i>et al.</i> 2013
	HM485353	isolate GB69	Italy	Bonito <i>et al.</i> 2013
	HM485354	isolate GB37	Italy	Bonito <i>et al.</i> 2013
<i>Tuber foetidium</i> Vittad.	AJ557543	isolate B-2452	Hungary: Garé	Halasz <i>et al.</i> 2005
	JQ288907	voucher ZB516	unknown	Unpublished
<i>Tuber oligospermum</i> (Tul. & C. Tul.) Trappe	KF021624	voucher CMI-UNIBO 4230	Morocco: Rabat, Marmora forest	Boutahir <i>et al.</i> 2013
<i>Tuber rapaeodorum</i> Tul. & C. Tul. *	KF021622	voucher CMI-UNIBO 4234	Morocco: Rabat, Marmora forest	Boutahir <i>et al.</i> 2013
	EU784429	voucher RBG Kew K(M)128884	England	Brock <i>et al.</i> 2009
<i>Tuber panniferum</i> Tul. & C. Tul.	DQ011849	voucher CMI-UNIBO 2483	Armenia: Dilijan	Unpublished
	AF132507	<i>Tuber panniferum</i>	unknown	Roux <i>et al.</i> 1999
	HM485380	isolate JT12835	Spain	Bonito <i>et al.</i> 2010
<i>Tuber rufum</i> Pollini	AY112894	<i>Tuber rufum</i>	unknown	lotti <i>et al.</i> 2002
	JF926123	isolate S90	Germany: Baden-Württemberg	Stobbe <i>et al.</i> 2012
	EF362475	voucher 1785	Italy	lotti <i>et al.</i> 2007
<i>Tuber indicum</i> Cooke & Massee	DQ329364	isolate Tind-hl01	China: Huili, Sichuan province	Wang <i>et al.</i> 2006a
	DQ375496	isolate Tind-hl02	China: Huili, Sichuan province	Wang <i>et al.</i> 2006b
	DQ375490	isolate Tind-gs01	China: Gongshan, Yunnan province	Wang <i>et al.</i> 2006b
	DQ375491	isolate Tind-gs05	China: Gongshan, Yunnan province	Wang <i>et al.</i> 2006b

orange, brown tone) in relation to season, habitat or state of ripeness, with abundant ramified and thin, sterile, white veins not discolouring when exposed to the air.

#### Odor

Weak, similar to stigmas of maize in the whitish gleba of not quite mature specimens, then more and more intense

and pleasant like the flavour, which resembles that of nuts, mainly hazelnuts.

*Peridium*

200–480 µm thick, exoperidium pseudoparenchymatous (100–180 µm), almost opaque in section, composed of globose-polygonal cells, 7–20 µm wide, with reduced cell lumen and deep brown walls up to 4 µm thick and fused with those of adjacent cells; endoperidium 100–300 µm thick, pseudoparenchymatous in the interior part of the endoperidium, the cells hyaline and smaller with a confusing pattern in contact with a layer of intertwined thin (2–3 µm) hyphae that extend into sterile veins of the gleba.

*Asci*

Globose to subglobose, 60–120 × 55–85 µm, with walls usually up to 3 µm thick, rarely to 10 µm, shortly stalked or sessile, 60–100 × 50–80 µm, with basal crozier, containing 1–6 spores.

*Ascospores*

Subglobose to broadly ellipsoid, Q 1.21–1.37, 20–45 × 18–35 µm excluding ornamentation, inversely proportional in size to the number of spores in the ascus, light brown reticulate, meshes 4–10 µm wide × 3–7 µm high, 3–5 across the spore width and often with an incomplete secondary crest inside, and with the top of the ornamentations sometimes hooked at the apex of the reticular knots.

*Endosporium*

Often two-layered in KOH.

*Glebal*

Hyphae hyaline, 2–8 µm wide.

HABITAT. — Europe from southern Europe to Ireland, Great Britain and Sweden (Gotland Isle) and East to the Caucasus Mountains, North Maghreb (rare), near and north middle Asia to Turkmenistan/Iran borders. Under broadleaved and coniferous trees and shrubs; its ascomata develop a bit throughout the year, commonly from summer to late autumn (Molinier *et al.* 2016; unpublished data).

NOMENCLATURE AND TAXONOMY

Although the nomenclature of *Tuber aestivum* is seriously compromised due to its frequent use in the wrong sense, the name *Tuber aestivum* proposed by Vittadini (1831) clearly connected to Micheli's taxon (1729). It is so well characterized morphologically that past mycologists and traders have always preferred it to valid antecedent synonyms such as *Tuber blotii* Eudes-Desl. (1824), *Mémoires de la Société Linnéenne de Calvados* 1824: 47 (1824) [MB 102874] (Maire 1930).

However, the early legitimate homonym *Tuber aestivum* (Wulfen) Spreng. 1827 [MB 218548] exists; consequently *Tuber aestivum* Vittad. is illegitimate (Art. 53.1). The basionym of this earlier homonym is *Lycoperdon aestivum* Wulfen, in Jacquin, *Collnea bot.* 1 (2): 349 (1787) [1786] [MB 162907]. The Wulfen's description (Appendix 3) clearly identifies the fungus as a *Rhizopogon* for which there is the

sanctioned name *Rhizopogon aestivus* (Wulfen) Fr. (1823). Tulasne & Tulasne (1851) included *Rhizopogon aestivus* as a synonym of their *Rhizopogon rubescens*, whose current name is *R. roseolus* (Corda) Th.Fr., and as such sometimes subsequently considered to include as synonymous the names *Lycoperdon aestivum* or *Tuber aestivum* (Wulfen) Spreng. (Saccardo 1888). Martin (1996) examined all the specimens labeled as *R. aestivus* present in the main European herbaria, determining them as *R. roseolus*.

Wulfen himself concludes that it is not the species described by Micheli both for size and for the very black external surface with large pyramidal warts, smell and taste. In fact, *Lycoperdon aestivum* had a smooth peridium, furfuraceous at best, thin and often cracked, whitish then reddish, like a potato, slightly brownish and finally blackish [*extus sordide albidum, & ex albido obsolete subrubescens, instar pomorum terrae (Solanum tuberosum L.), dein dilute fuscens, nigrans denique; glabrum... cuticula tenerrima, hinc inde disrupta, & exstans, squamules exhiberet furfuraceus...*], spongy gleba cellulose-porous at first white then almost ashy, brownish, blackish (*Caro intus spongiosa, subcoriacea, prima alba, tum obsolete, & triste ex albido subcinerascens, fuscens, subnigricans... celluloso-porosa*), without marked smell and without taste (*Odor recentium nullus, aut certe debilissimus & subnauseosus. Sapor nullus, nisi imaginarius*).

No specimens of this species have been found in the herbaria of Wulfen (W), Fries (UPS) and KPJ Sprengel (VH/de), for this reason we selected as neotype of *Lycoperdon aestivum* a specimen of *Rhizopogon roseolus* collected in Carinthia. (Austria), place of origin of *Lycoperdon aestivum*.

A proposal will be written to conserve the name *Tuber aestivum* Vittad. and reject the legitimate early homonym *Tuber aestivum* (Wulfen) Spreng. and the early synonym *Tuber blotii* Eudes-Desl.

The proposal to retain the name *Rhizopogon roseolus* (Corda) Th. Fr. against *Rhizopogon aestivus* (Wulfen) Fr. should also be considered.

NEOTYPE OF *LYCOPERDON AESTIVUM* WULFEN. — Austria. Carinthia, St. Mergereten, Gotschich, leg. Irmegard Krisai Greilhuber, 9.IX.1998, sub nomine *Rhizopogon roseolus* (neo-, WU[WU 25744], here designated; MycoBank Typification number: MBT 10001892).

*Tuber borchii* Vittad.  
(Figs 2; 4B; 5C, D)

*Monographia Tuberacearum*: 44 (1831).

MYCOBANK NUMBER. — MB 118774.

GENBANK. — MZ423174 (nrITS), MZ458418 (nr β-tubulin), MZ458422 (nrEF 1-α).

LECTOTYPE OF *TUBER BORCHII* VITTAD. — Vittadini 1831 tab. I (presented with the indication XX), fig. III E-F here reprinted in Fig. 2. (here designated; MycoBank Typification number: MBT 10001893).

EPITYPE OF *TUBER BORCHII* VITTAD. — Italy. Lombardy, Varzi, Castello Oramala, sub *Quercus pubescens* Willd., 6.III.2019, 44°50'28"N,



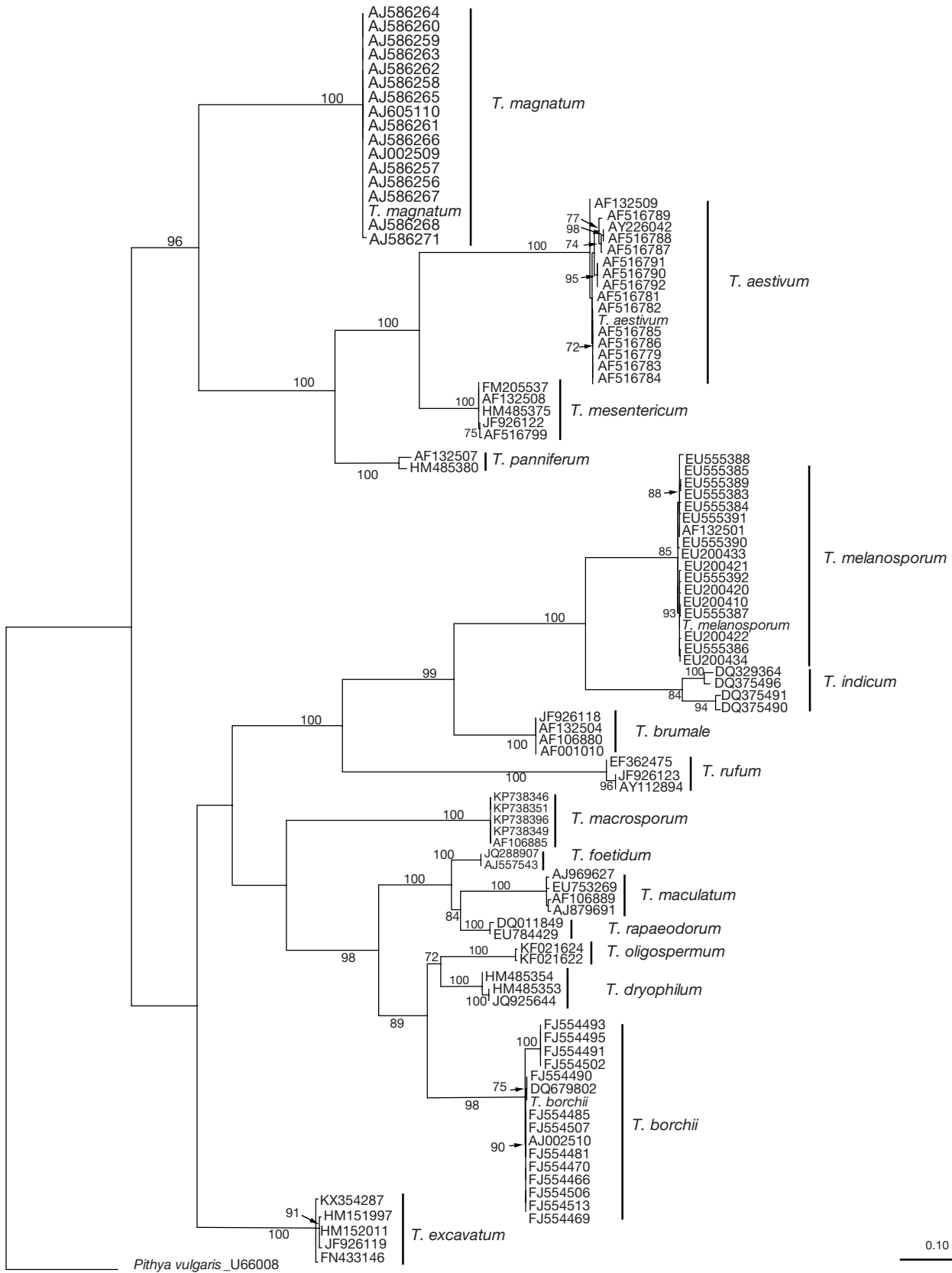


FIG. 1. — Maximum likelihood tree obtained from the alignment of ITS nuclear rDNA region sequences showing relationships among *Tuber* taxa and types. Sequences obtained during this study are indicated in bold. Bootstrap values  $\geq 70\%$  are indicated on the nodes of branches. The scale indicates the number of substitutions per site. *Pithya vulgaris* Fuckel was included as outgroup.

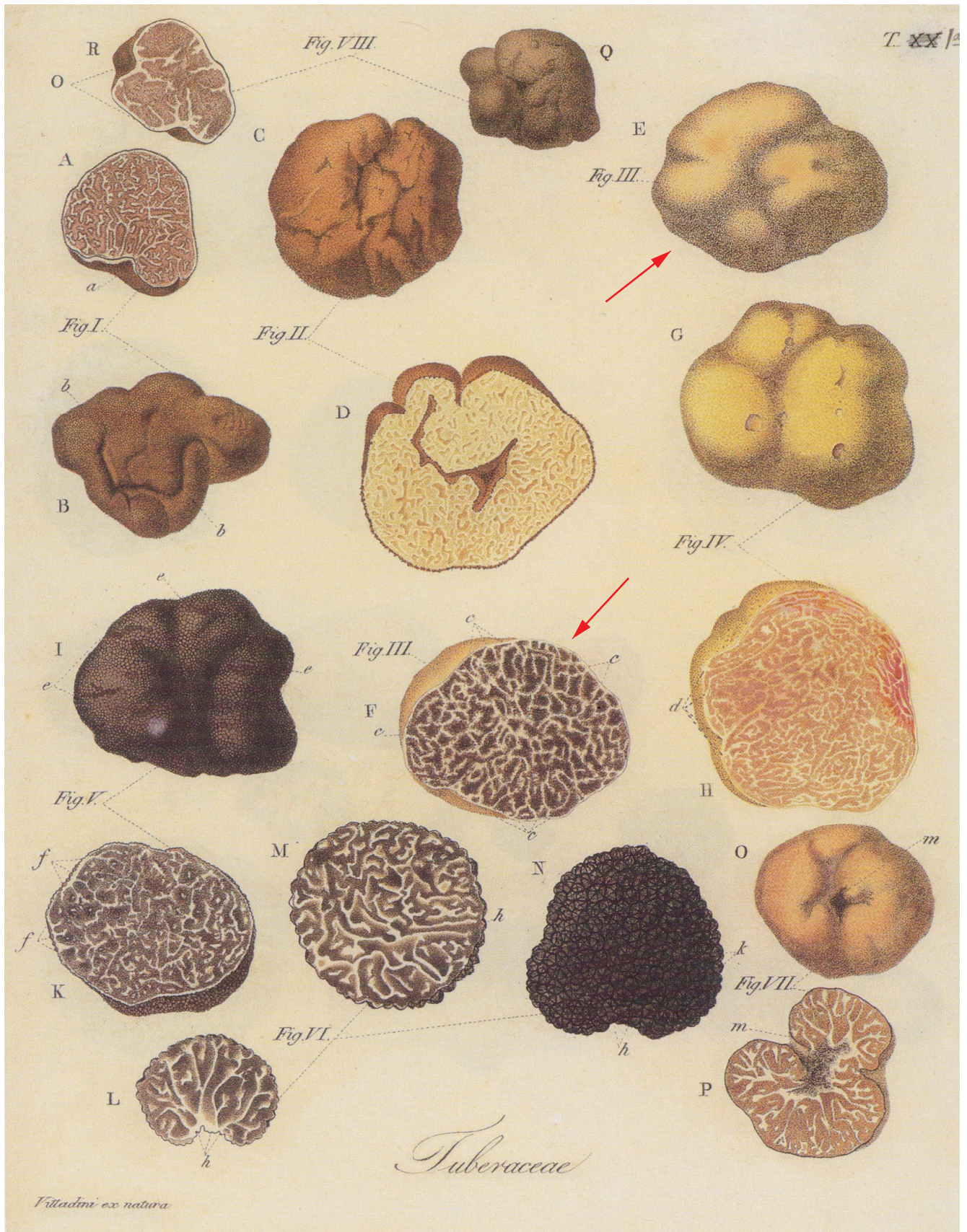


FIG. 2. — Table I (XX), lectotype of *Tuber borchii* Vittad. (red arrowed).

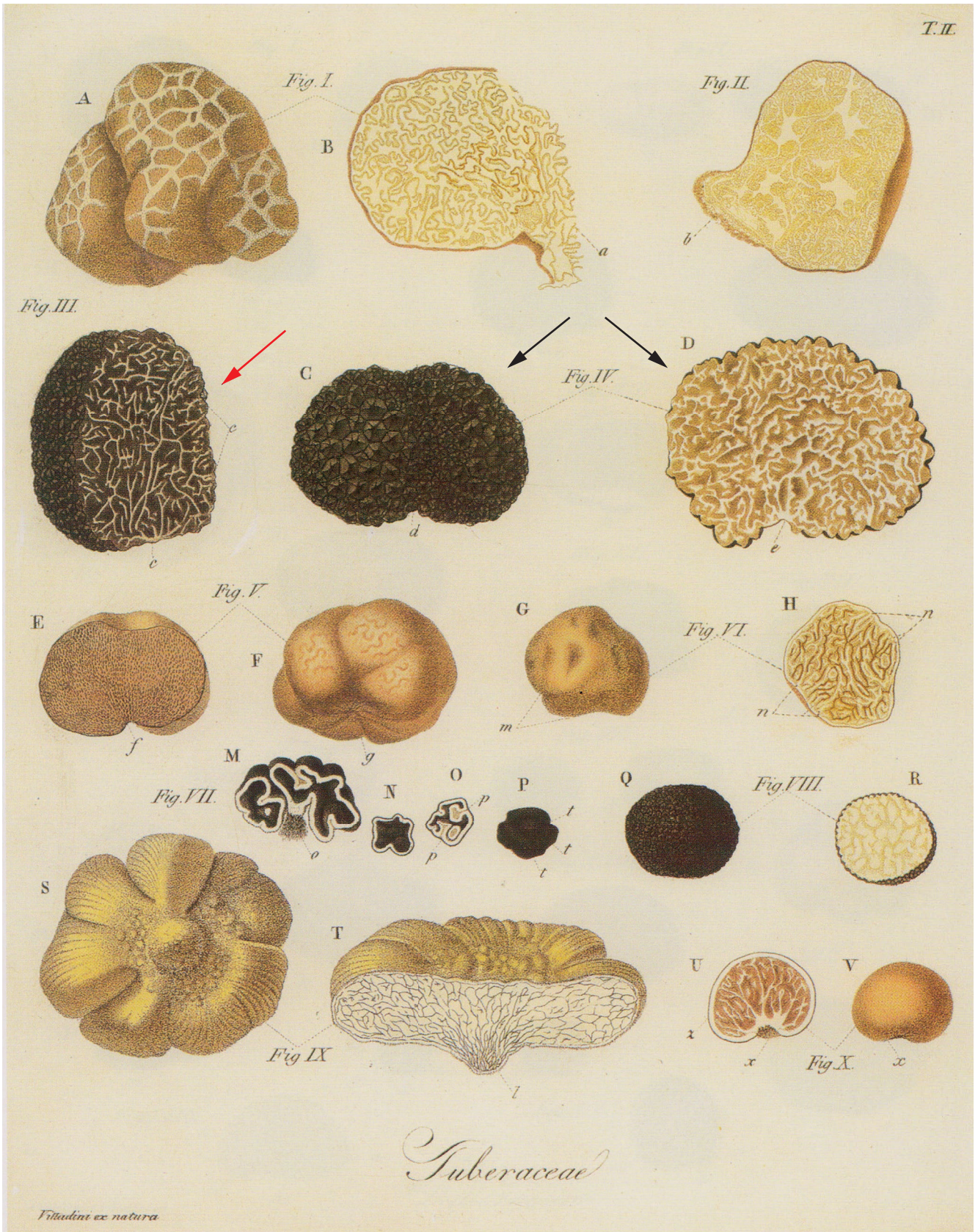


FIG. 3. — Table II, lectotypes of *Tuber melanosporum* Vittad. (red arrowed) and *Tuber aestivum* Vittad. (black arrowed).



FIG. 4. — Ascomata of A) epitype of *Tuber aestivum* Vittad. (AQUI 10150); B) epitype of *T. borchii* Vittad. (AQUI 10151); neotype of *T. magnatum* Vittad (TO HG3458); epitype of *T. melanosporum* Vittad. (AQUI 10152). Scales bars: 1 cm.

9°11'32"E, twenty ascomata, leg. Stefano Seghezzi, det. Giovanni Pacioni (epi-, AQUI[AQUI10151] here designated MycoBank Typification number: MBT 10001895).

#### DESCRIPTION

##### *Ascomata*

Hypogeous globose, lobate or irregular, up to 7 cm in diameter, rarely larger, sometimes with a flat base without excavation, surface of peridium pubescent, whitish (FCF3CF light grayish yellow, F9E79F very soft yellow, F0B27A soft orange), yellowish-gray, ocher, brownish, sometimes with reddish spots, smooth when fully mature, still pubescent in the depressions.

##### *Gleba*

Firm from whitish (D5DBdB light grayish green, EC7063 soft red, 797D7F dark grayish blue) to ocher with reddish hues, becoming greyish or reddish-brown by maturity, with ramified-anastomoses and thin sterile veins whitish in youth. Later ochraceous or reddish, marbled with wide, anastomosing veins which arise from various places of the peridium.

##### *Odor*

Pleasant, then strongly garlic sulfurous; taste strong.

##### *Peridium*

150-600 µm thick, exoperidium pseudoparenchymatous, 100-300 µm thick with *textura globosa*, formed of swollen cells ranging from suglobose to irregularly ellipsoid 10-50 × 8-24 µm, yellowish but strongly coloured on the surface, with walls of collenchymatic type thickened up to 4 µm, surface with tapered hyaline thick-walled, one to three celled cystidia up to 100 µm long, up to 8 µm wide at the inflated base; endoperidium plectenchymatous 60-450 µm wide with intertwined, mainly periclinal, hyaline hyphae 20-24 × 5-6 µm, with thin walls, mixed with a few rounded cells with slightly thickened walls; sterile veins emerge from the endoperidium to penetrate the gleba from various parts of the peridium.

##### *Asci*

Globose to subglobose, 60-100 × 50-80 µm, with walls 1-1.5 µm to 5 µm thick, sessile or short stalked, croziered at the foot, containing 1-4 spores.

##### *Ascospore*

Subglobose to broadly ellipsoid, Q 1.06-1.46, 20-55 × 18-42 µm excluding ornamentation, inversely proportional in size to the number of spores in the ascus, at first translucent, then becoming light yellow to yellowish brown, sometimes with

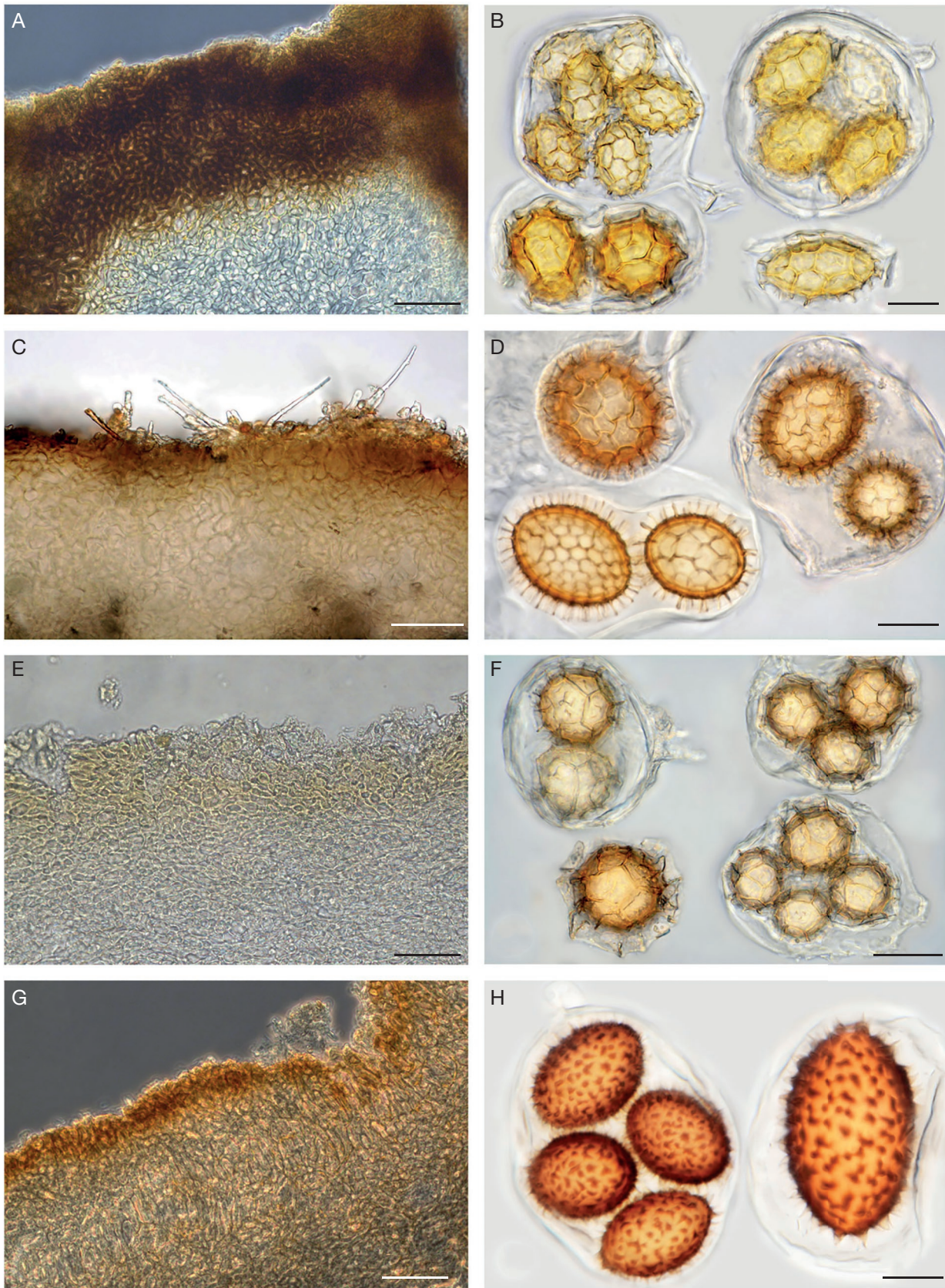


FIG. 5. — Cross sections of peridia, asci and spores **A, B**, *Tuber aestivum* epitype; **C, D**, *T. borchii* epitype; **E, F**, *T. magnatum* neotype; **G, H**, *T. melanosporum* epitype. Scales bars A, C, E, G, 50  $\mu$ m; B, D, F, H, 20  $\mu$ m.

a reddish hue, regularly alveolate with mostly hexagonal but sometimes rectangular or elongated alveolae numbering 4-11 along the spore length and 3-9 across the spore width, 4-10 µm wide but usually near 7 µm, and 3-5 µm high.

#### *Endosporium*

Often two-layered in KOH.

#### *Glebal*

Hyphae hyaline, 4-10 × 20-32 µm

HABITAT. — Species naturally present in all Europe to the southern Scandinavian peninsula, from Portugal to the Caucasus and Anatolia, under broadleaved and coniferous trees, mainly sandy soil, commonly from late autumn to late spring (Ceruti *et al.*, 2003; unpublished data)

#### NOMENCLATURE AND TAXONOMY

There may be a precedent synonym for this species: *Tuber albidum* Picco, *Melethemata inauguralia*: 79 (1788) [MB 227650] which however is unavailable, unpriorable, because of the sanctioned homonym *Tuber albidum* Fr. (1823) [MB 227428], which refers to a different species. This Fries' taxon is difficult to identify because of its diagnosis "*verrucis exasperatum, albidum*". Vittadini (1831: 40, Obs.II) wrote about it: 'the whitish external color in warty *Tuber* is unknown to me, I suspect. So the (species) named *albidum* is doubtful. (Obs. II. *Tuber albidum* Fr. ... *Color externus albidus in Tuberculosis muricatis mihi prorsus extraneus, suspectus. Hinc albidum nomen ambiguum.*)'. Perhaps due to a '*lapsus calami*', *Tuber albidum* Fr. is a questionable species that does not even today find a match among the known species of *Tuber* where all the warty peridium species are black or deep brown (Bonito *et al.* 2013).

Fries (1823: 291) claims to have examined a dried sample (vs, vidi siccam), but no specimen of *Tuber albidum* was found in the Herbarium of Fries (UPS-FRIES) or a drawing of this taxon among Fries documents.

From the Fries' description it is understood that it could refer to *Tuber aestivum* and two references (Cesalpino 1583: 613, and Micheli 1729: 221) could confirm it, while a third (Gleditsch 1753: 257) '*Lycoperdon globosum, subterraneum ...*' could describe a *Rhizopogon*.

In this situation, the most appropriate solution is to propose the rejection of the name *Tuber albidum* Fr. in application of Art. 56.1 of the Shenzhen Code (Turland *et al.*, 2018) and a proposal will be made to that effect.

The older name *Tuber albidum* Picco (1788) in addition to being unavailable (Mycobank <http://www.mycobank.org/Biolomics.aspx?Table=Mycobank&Rec=28125&Fields=All>) is a poorly defined and variously interpreted *Tuber* species that can include several whitish species such as *T. maculatum* Vittad., *T. rapiodorum* Tul. & C.Tul., *T. dryophilum* Tul. & C.Tul. and *T. puberulum* Berk. & Broome, etc. (Halász *et al.* 2005). The previous neotypification of *T. borchii* proposed by Mello *et al.* (2000) cannot be accepted because of formal and substantive reasons: 1) presence of an existent possible lectotype [tab. I, fig.III E-F in Vittadini

(1831) here designated]; 2) the probable loss of the three collections indicated as 'neotype', lacking however of any collecting data. They were sent by Vittadini to the Tulasne brothers (Tulasne & Tulasne 1851: 146) and preserved originally in PC (Cryptogamic Herbarium of Muséum National d'Histoire Naturelle, Paris), and later retained in TO (Mattirollo's Herbarium); 3) the fact that the proposed neotype was not sequenced.

Hence there is a need to propose an epitype using a specimen with well-defined collection data and molecular characterization.

Bonuso *et al.* (2010) hypothesized the presence of two cryptic species (referred to as haplotype 1 and haplotype 2) within Italian populations of *T. borchii*. The *T. borchii* epitype designated here belongs to haplotype 1.

#### *Tuber magnatum* Picco (Figs 4C; 5E, F)

*Melethemata inauguralia*: 79 (1788).

*Tuber griseum* Borch ex Pers., *Synopsis Methodica Fungorum*: 127 (1801); sanct. Fr., *Systema Mycologicum* 2 (2): 292 (1823). (MB 179335).

MYCOBANK NUMBER. — MB 184470.

GENBANK. — MZ423175 (nrITS), MZ458419 (nr β-tubulin), MZ458423 (nrEF 1-α).

NEOTYPE OF *TUBER MAGNATUM* PICCO.— Italy, Piedmont, Montechiaro D'Asti, Loc. Seria, 45°00'30"N, 8°06'45"E, lowland grove with *Quercus robur* L. and *Populus tremula* L., under *Q. robur*, 29.IX.2019, four ascomata, leg. Pino Panzini, det. Alfredo Vizzini (neo-, TO[HG3458] here designated; MycoBank Typification number: MBT 10001896).

#### DESCRIPTION

##### *Ascomata*

Hypogeous, globose to lobate or flat, up to 10-15 cm broad and more rarely with a detachable basal mycelial cluster. Peridium whitish (FFFFFF0 ivory), yellowish, pale ocher (FFD700 gold, DAA520 goldenrod) more or less greyish (DCDCDC gainsboro, D3D3D3 light gray) sometimes with greenish or reddish hues; smooth surface finely grainy under a lens.

##### *Gleba*

Whitish (FFFFFF0 ivory), yellowish or pale ocher, brownish (8B4513 saddle brown, A0522D sienna, F2F2F2) with greyish tones, often with reddish purple spots; veins thin, whitish, branched, anastomosing, sometimes forming white gangliform thickenings.

##### *Odor*

Pleasant, penetrating, of garlic or strong cheese; taste pleasant.

##### *Peridium*

200-500 µm thick, entirely pseudoparenchymatous basically of globular cells; exoperidium 80-110 µm in the outer

layer with globose cells having walls up to 4 µm thick and slightly yellow; peridial surface with infrequent stocky, cylindrical-clavate dermatocystidia 14–20 × 8–10 µm; endoperidium 120–380 µm thick, of thin-walled globose and polygonal cells 6.0–36 × 6–23 µm with rare cylindrical cells 6–9 × 14–20 µm.

#### *Asci*

Globose, short-stalked or sessile, 60–90 × 40–70 µm (Fig. 2F), with walls up to 4 µm thick and a basal crozier, containing 1–4 spores.

#### *Ascospores*

Alveolate, globose to broadly ellipsoid, Q 1.05–1.33, 20–50 × 15–42 µm excluding ornamentation, inversely proportional in size to the number of spores in the ascus, yellowish, pale ocher or sometimes light yellow-brown; reticulum with 1–3 regular or coarsely irregular, 3–6 sided alveoli 10–22 µm wide and 4–5 (–8) µm high, sometimes with ridges within the alveolae.

#### *Endosporium*

Often two-layered in KOH.

#### *Gleba*

Hypae hyaline, 4–8 µm broad, in the external veins with cylindrical or clavate-elongated cells mixed with subglobose cells 5–42 × 4–16 µm.

HABITAT. — moist (mesophilic) woods mainly with broad-leaved trees and conifers such as *Abies alba* Mill., on sandy-silty soils in southern Europe (Italy and a few neighbouring French and Swiss areas, the Balkan-Pannonia region, Northern Anatolia), from late summer to early winter (Belfiori *et al.* 2020; unpublished data). It has also been reported in Thailand, but vouchers have not been made available (Suwannarach *et al.*, 2017).

#### NOMENCLATURE AND TAXONOMY

The name most frequently used for this species is *Tuber magnatum* Picco (1788) for which a neotype is herein designated. However, a competing name exists, namely *Tuber griseum* Borch ex Pers., validated by Persoon (1801) and sanctioned by Fries (1823), for which a lectotype and epitype are also herein designated. The epitype is represented by a *T. magnatum* with a greyish peridium.

Borch's or Picco's authentic specimens were not available for either *T. griseum* or *T. magnatum*.

LECTOTYPE OF *TUBER GRISEUM*. — Truffe grise De Borch, Lettres sur les Truffes du Piémont, 1<sup>st</sup> figure (1780); here reprinted in Appendix 4 (here designated; MycoBank Typification number: MBT 10001897).

EPITYPE OF *TUBER GRISEUM*. — Italy. Piedmont, Monte Magno, 44°59'02"N, 8°19'35"E, under *Quercus robur*, 07.XII.1998, one fragment, leg. Virgilio Gavazza, det. Alfredo Vizzini (epi-, TO[HG 3557] here designated; MycoBank Typification number: MBT 10001898).

A proposal will be written to conserve the name *T. magnatum* and reject the sanctioned name *T. griseum*.

### *Tuber melanosporum* Vittad.

(Figs 2D; 3G, H; Appendix 2)

*Monographia Tuberacearum*: 36 (1831).

*Tuber nigrum* Bull., *Herbier de la France* 8: t.356 (1788). (MB 204568).

MYCOBANK NUMBER. — [MB 192144](#).

GENBANK. — [MZ423176](#) (nrITS), [MZ458420](#) (nr β-tubulin), [MZ458424](#) (nrEF 1-α).

LECTOTYPE OF *TUBER MELANOSPORUM* VITTAD. — Vittadini 1831: tab. II fig. III; here reprinted in Fig. 3. (here designated; MycoBank Typification number: MBT 10001899).

EPITYPE OF *TUBER MELANOSPORUM* VITTAD. — Italy. Lombardy, Monza, Parco Villa Reale, 1.II.2019, sub *Carpinus betulus* and *Tilia cordata*, 45°35'39"N, 9°16'25"E, six ascomata, legit Stefano Seghezzi, det. Giovanni Pacioni (epi-, AQUI[AQUI 10152] here designated; MycoBank Typification number: MBT 10001900).

Authentic Vittadini specimens examined: K(M) 254905, 254904 e 254904, TO e UPS (F-628213).

#### DESCRIPTION

##### *Ascomata*

Hypogeous, globose or irregular, sometimes lobed, rarely more than 10 cm broad, surface of peridium reddish brown then reddish black or brownish black (#330000 very dark red), covered with pyramidal warts 2–5 mm at the base and 0.5–2.5 mm tall, depressed at the apex and with 4–6 sides joined with radial edges.

##### *Gleba*

Firm and compact, from reddish-gray to black-purple or black with violet hues (3B2512–3B3938 dark shade orange to 26211D–2B2017 very dark orange shade); sterile veins thin, much branched, whitish or reddish when exposed to the air.

##### *Odor*

Intense, complex, pleasant, the taste with a bitter aftertaste.

##### *Peridium*

300–450 µm thick; exoperidium pseudoparenchymatous, 70–15 µm thick, the outer layer 40–80 µm thick, of globose to sub-globose sclerenchymatous pigmented cells, with very thickened walls and the cellular lumen reduced or absent, 12–20 × 7–12 µm, limited to the superficial part of the warts underlaid by a palisade layer 33–65 µm thick of elongated cells (16–26 × 5–6.5 µm) with the major axis perpendicular to the surface just below the exoperidium; endoperidium 240–350 µm thick, pale with a pseudoparenchymatous mixture of cells 4–11 × 2–7 µm and ranging from subglobose, cuboid, polygonal, cylindric or irregularly swollen, elongated at one end, pale with collenchymatic walls 2–3 µm thick. The palisade structure is not always visible, depending on the cut, generally within the outermost layer of sclerenchymatous cells lies a homogeneous pseudoparenchymatous layer of endoperidium.

### Asci

Globose to subglobose, 90-145 × 70-125 µm, with walls up to 4 µm, sometimes thicker in the final stages VI of ripening (Zarivi *et al.* 2014), short stalked or thick, sessile with a crozier at the base; containing 1-6 spores.

### Ascospores

Spiny, ellipsoid, Q 1.28-1.56, 20-56 × 14-36 µm excluding ornamentation, inversely proportional in size to the number of spores in the ascus, opaque when ripe, intense brown, blackish, with black-dark brownish spines, short, robust and rigid, sometimes curved, 1.5-3.0 (-4) µm long to 2 µm wide at the base, usually separated but sometimes connected at the base to form short crests, occurring at a density of 11-13 spines per 100 µm<sup>2</sup>.

### Gleba

Hyphae hyaline, 14-36 × 6-8 µm, at full maturity brownish.

HABITAT. — mainly under broadleaf trees and shrubs, especially thermophilic oaks, but also European hop-hornbeam (*Ostrya carpinifolia*) and hazelnuts (*Corylus avellana*), rare under pines or other conifers. Its natural environments are extended in calcareous soils in southern Europe from the Iberian Peninsula to France and Italy, rarer in the Balkans and western Anatolia, late autumn and winter (Le Tacon 2017).

### NOMENCLATURE AND TAXONOMY

The first name certainly assigned to this species was *Tuber nigrum* by Bulliard (Herbier de la France 8: t. 356, 1787-1788), however Bulliard (1791) later used the name “*Tuber cibarium*” (Histoire des champignons de la France. I: 74) referring to plate 356 of the Herbier de France. *Tuber cibarium* Bull., nom. illegit., was applied to a taxon that included all species of *Tuber* with warty black peridium. The epitype of *Tuber nigrum* is represented by a *T. melanosporum* although Tab. 356, in our possession, depicts a specimen with gray-brown gleba like *T. brumale*. (Appendix 5). However, chromatic fidelity cannot be expected from a 1788 print.

Vittadini recognized the characteristic blackish spiny spores, thereby differentiating it from *T. brumale* Vittad., and from the other warty black peridium species.

LECTOTYPE OF *TUBER NIGRUM* BULL. — (here designated; MycoBank Typification number: MBT 10001901: t.356 (Bulliard 1787-1788)). here reprinted in Appendix 5.

EPITYPE OF *TUBER NIGRUM* BULL. — France. Lot 46090 Bellefont-La Rauze 44°30'39"N, 1°30'47"E, *Q. pubescens*, 14.XII.2019, leg. Pierre Sourzat, det. Giovanni Pacioni (epi-, AQUI[AQUI 10208] here designated; MycoBank Typification number: MBT 10001902).

A proposal will be written to conserve the name *T. melanosporum* and reject the early legitimate synonym *T. nigrum*.

### CONCLUSION

These four species and their type names are applied to the most economically important species of truffles and our typifications provide a taxonomic reference to them. In addition,

these typifications of the names of the four truffle species will allow us to propose conservation of the name *Tuber aestivum* Vittad. and reject the homonym and the earlier competing names *Tuber aestivum* (Wulfen) Spreng. and *Tuber blotii* Eudes-Desl., conservation of *T. magnatum* Picco against *Tuber griseum* Borch ex Pers. sanct. Fries (1823), conservation of *T. melanosporum* Vittad. against *Tuber nigrum* Bull. in accordance with Art. 14.1 of Shenzhen Code (Turland *et al.* 2018). These conservations prevent destabilization of names of mushrooms of economic interest, but they needed the typification of species concerned. *Tuber albidum* Picco does not put the name *T. borchii* Vittad. at risk, because the sanctioned homonym *Tuber albidum* Fr. (1823) refers to a different species. Their accurate morphological and molecular characterization based on type specimens clearly define these names for future taxonomic and phylogenetic investigations.

### Acknowledgements

We are strongly grateful to Stefano Seghezzi for providing specimens of *Tuber aestivum*, *T. borchii* and *T. melanosporum* from the Lombard areas frequented by Carlo Vittadini. Giovanni Pacioni wishes to express his gratitude to and to Rossella Marcucci (PD) and Åsa Krus (UP), and the mourned R.G.W. Dennis (K) and A. Ceruti (TO), for allowing him to study the original collections of Vittadini and Irmegard Greilhuber (WU) for her support in the neotypification of *Lycoperdon aestivum* Wulfen. A special thanks to Begoña Aguirre-Hudson (K) for advising us in advance about the new status of the Vittadini collections in the Fungarium catalog, and to Amy Rossman for checking our nomenclature notes and her invaluable advice. Our deepest gratitude to Scott Redhead and Shaun Pennycook who have greatly improved earlier versions of this manuscript.

### REFERENCES

- BELFIORI B., D'ANGELO V., RICCIONI C., LEONARDI M., PAOLOCCI F., PACIONI G. & RUBINI A. 2020. — Genetic structure and phylogeography of *Tuber magnatum* populations. *Diversity* 12: 44. <https://doi.org/10.3390/d12020044>
- BENUCCI G. M. N., CSORBAI A. G., BACIARELLI FALINI L., MAROZZI G., SURIANO E., SITTA N. & DONNINI D. 2016. — Taxonomy, Biology and Ecology of *Tuber macrosporum* Vittad. and *Tuber mesentericum* Vittad., in ZAMBONELLI A., IOTTI M., MURAT C. (eds) *True Truffle (Tuber spp.) in the World. Soil Biology*, vol 47. Springer, Cham. [https://doi.org/10.1007/978-3-319-31436-5\\_5](https://doi.org/10.1007/978-3-319-31436-5_5)
- BONITO G. M., GRYGANSKYI A. I. P., TRAPPE J. M. & VILGALYS R. 2010. — A global meta-analysis of *Tuber* ITS rDNA sequences: species diversity, host associations and long-distance dispersal. *Molecular Ecology* 19: 4994-5008 <https://doi.org/10.1111/j.1365-294X.2010.04855.x>
- BONITO G., SMITH M. E., NOWAK M., HEALY R. A., GUEVARA G., CÁZARES E., KINOSHITA A., NOUHRA E. R., DOMÍNGUEZ L. S., TEDERSOO L., MURAT C., WANG Y., MORENO B. A., PEISTER D. H., NARA K., ZAMBONELLI A., TRAPPE J. M. & VILGALYS R. 2013. — Historical biogeography and diversification of truffles in the Tuberales and their newly identified southern hemisphere sister lineage. *PLoS ONE* 8: e52765. <https://doi.org/10.1371/journal.pone.0052765>



- BONUSO E., ZAMBONELLI A., BERGEMANN S. E., IOTTI M. & GARBELOTTO M. 2010. — Multilocus phylogenetic and coalescent analyses identify two cryptic species in the Italian bianchetto truffle, *Tuber borchii* Vittad. *Conservation Genetics* 11: 1453-1466. <https://doi.org/10.1007/s10592-009-9972-3>
- BOUTAHIR S., IOTTI M., PIATTONI F. & ZAMBONELLI A. 2013. — Morphological and molecular characterization of *Tuber oligospermum* mycorrhizas. *African Journal of Agricultural Research* 8: 4081-4087. <https://doi.org/10.5897/AJAR2013.7354>
- BROCK P. M., DÖRING H. & BIDARTONDO M. I. 2009. — How to know unknown fungi: the role of a herbarium. *New Phytologist* 181: 719-724. <https://doi.org/10.1111/j.1469-8137.2008.02703.x>
- BULLIARD P. 1787-88. — *Herbier de la France*: vol 8: Pl. 356. Paris.
- BULLIARD P. 1791. — *Histoire des champignons de la France*. Imprimerie de la Société typographique, Paris. 368 p. <http://catalogue.bnf.fr/ark:/12148/cb45211528q>
- CERUTI A., FONTANA A. & NOSENZO C. 2003. — Le Specie Europee del Genere Tuber. Museo Regionale di Scienze Naturali, Torino.
- CESALPINO A. 1583. — De Plantis Libri XVI. Giorgio Marescotti, Firenze.
- FRIES E. 1823. — *Systema Mycologicum sistens fungorum ordines, genera et species*. Officina Berlingiana, Lundae. <https://doi.org/10.5962/bhl.title.5378>
- GARDES M. & BRUNS T. D. 1993. — ITS primers with enhanced specificity for basidiomycetes application to the identification of mycorrhizae and rusts. *Molecular Ecology* 2: 113-118. <https://doi.org/10.1111/j.1365-294X.1993.tb00005.x>
- GLASS N. L. & DONALDSON G. C. 1995. — Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous ascomycetes. *Applied and Environmental Microbiology* 61: 1323-1330.
- GLEDITSCH J. G. 1753. — Methodus fungorum exhibens genera, species et varietates cum caractere. Sumtibus Scholae Realis, Berlin.
- INTERNATIONAL CODE OF BOTANICAL NOMENCLATURE. 1983. — VOSS E. G. (ed.). Bohn, Scheltema & Holkema, Utrecht.
- HALÁSZ K., BRATEK Z., SZEGŐ D., RUDNÓY S., RÁCZ I., LÁSZTITY D. & TRAPPE J. M. 2005. — Tests of species concepts of the small, white, European group of *Tuber* spp. based on morphology and rDNA ITS sequences with special reference to *Tuber rapaeodorum*. *Mycological Progress* 4: 281-290. <https://doi.org/10.1007/s11557-006-0132-6>
- INTERNATIONAL CODE OF BOTANICAL NOMENCLATURE. 1983. — VOSS E. G. (ed.). Bohn, Scheltema & Holkema, Utrecht.
- IOTTI M., AMICUCCI A., STOCCHI V. & ZAMBONELLI A. 2002. — Morphological and molecular characterization of mycelia of some *Tuber* species in pure culture. *New Phytologist* 155: 499-505. <https://doi.org/10.1046/j.1469-8137.2002.00486.x>
- IOTTI M., AMICUCCI A., BONITO G., BONUSO E., STOCCHI V. & ZAMBONELLI A. 2007. — Selection of a set of specific primers for the identification of *Tuber rufum*: a truffle species with high genetic variability. *FEMS Microbiology Letters* 277: 223-231. <https://doi.org/10.1111/j.1574-6968.2007.00963.x>
- KATO H. & STANDLEY D. M. 2013. — MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Molecular Biology and Evolution* 30: 772-780. <https://doi.org/10.1093/molbev/mst010>
- LEONARDI M., PAOLOCCI F., RUBINI A., SIMONINI G. & PACIONI G. 2005. — Assessment of inter- and intra-specific variability in the main species of *Boletus edulis* complex by ITS analysis. *FEMS Microbiology Letters* 243: 411-416. <https://doi.org/10.1016/j.femsle.2005.01.003>
- LEONARDI M., PAZ-CONDE A., GONZALO G., SALVI D. & PACIONI G. 2019. — Two new species of *Tuber* previously reported as *Tuber malacoderмум*. *Mycologia* 111: 676-689. <https://doi.org/10.1080/00275514.2019.1603777>
- LE TACON F. 2017. — *Les Truffes*. AgroParisTech: Nancy.
- MAIRE R. 1930. — Sur le *Tuber blotii* E. Deesl. *Bulletin Société Mycologique de France* 46: 149-150.
- MARTIN F., KOHLER A., MURAT C., BALESTRINI R., COUTINHO P. M., JAILLON O., MONTANINI B., MORIN E., NOEL B., PERCUDANI R., PORCEL B., RUBINI A., AMICUCCI A., AMSELEM J., ANTHOUARD V., ARCIONI S., ARTIGUENAVE F., AURY J. M., BALLARIO P., BOLCHI A., BRENN A., BRUN A., BUÉE M., CANTAREL B., CHEVALIER G., COULOUX A., DA SILVA C., DENOEUD F., DUPLESSIS S., GHIGNONE S., HILSELBERGER B., IOTTI M., MARÇAIS B., MELLO A., MIRANDA M., PACIONI G., QUESNEVILLE H., RICCIONI C., RUOTOLO R., SPLIVALLO R., STOCCHI V., TISSERANT V., VISCOMI A. R., ZAMBONELLI A., ZAMPIERI E., HENRISSAT B., LEBRUN M. H., PAOLOCCI P., BONFANTE P., OTTONELLO S. & WINCKER P. 2010. — Périgord black truffle genome uncovers evolutionary origins and mechanisms of symbiosis. *Nature* 464: 1033-1038. <https://doi.org/10.1038/nature088670052765>
- MARTIN M. P. 1996. — *The genus Rhizopogon in Europe*. Societat Catalana de Micologia: Barcelona, 173 p.
- MELLO A., GARNERO L., MEOTTO F. & BONFANTE P. 1998. — Specific primers for rapid typing of *Tuber borchii* mycorrhizal roots. *Acta Horticulturae* 457: 229-234. <https://doi.org/10.17660/ActaHortic.1998.457.28>
- MELLO A., VIZZINI A., LONGATO S., ROLLO F., BONFANTE P. & TRAPPE J. M. 2000. — *Tuber borchii* versus *Tuber maculatum*: neotype studies and DNA analyses. *Mycologia* 92: 326-331. <http://doi.org/10.1080/00275514.2000.12061163>
- MELLO A., CANTISANI A., VIZZINI A. & BONFANTE P. 2002. — Genetic variability of *Tuber uncinatum* and its relatedness to other black truffles. *Environmental Microbiology* 4: 584-594. <https://doi.org/10.1046/j.1462-2920.2002.00343.x>
- MELLO A., MURAT C., VIZZINI A., GAVAZZA V. & BONFANTE P. 2005. — *Tuber magnatum* Pico, a species of limited geographical distribution: its genetic diversity inside and outside a truffle ground. *Environmental Microbiology* 7: 55-65. <https://doi.org/10.1111/j.1462-2920.2004.00678.x>
- MELLO A., MURAT C. & BONFANTE P. 2006. — Truffles: much more than a prized and local fungal delicacy. *FEMS Microbiology Letters* 260: 1-8. <https://doi.org/10.1111/j.1574-6968.2006.00252.x>
- MELLO A., ZAMPIERI E. & ZAMBONELLI A. 2017. — Truffle ecology: genetic diversity, soil interactions and functioning, in VARMA A., PRASAD R. & TUTEJA N. (eds) *Mycorrhiza-Function, Diversity, State of the Art*. Springer, Cham, Switzerland: 231-252. [https://doi.org/10.1007/978-3-319-53064-2\\_11](https://doi.org/10.1007/978-3-319-53064-2_11)
- MICHELI P. A. 1729. — Nova plantarum genera iuxta Tournefortii methodum disposita quibus plantae 1900 recensentur. Bernardi Paperinii, Firenze.
- MOLINIER V., PETER M., STOBBE U. & EGLI S. 2016. — The Burgundy truffle (*Tuber aestivum* syn. *uncinatum*): a truffle species with a wide range over Europe. in ZAMBONELLI A., IOTTI M. & MURAT C. (eds) *True Truffles (Tuber spp.) in the World*. Soil Biology 47. Springer, Cham: Switzerland: 33-47. [https://doi.org/10.1007/978-3-319-31436-5\\_3](https://doi.org/10.1007/978-3-319-31436-5_3)
- MURAT C., VIZZINI A., BONFANTE P. & MELLO A. 2005. — Morphological and molecular typing of the below-ground fungal community in a natural *Tuber magnatum* truffle-ground. *FEMS Microbiology Letters* 245: 307-313. <https://doi.org/10.1016/j.femsle.2005.03.019>
- MURAT C., PAYEN T., NOEL B., KUO A., MORIN E., CHEN J., KOHLER A., KRIZSAN K., BALESTRINI R., DA SILVA C., MONTANINI B., HAINAUT M., LEVATI E., BARRY K. W., BELFIORI B., CICHOCKI N., CLUM A., DOCKTER R. B., FAUCHERY L., GUY J., IOTTI M., LE TACON F., LINDQUIST E. A., LIPZEN A., MALAGNAC F., MELLO A., MOLINIER V., MIYAUCHI S., POULAIN J., RICCIONI C., RUBINI A., SITRIT Y., SPLIVALLO R., TRAEGER S., WANG M., ZIFCAKOVA L., WIPF D., ZAMBONELLI A., PAOLOCCI F., NOWROUSIAN M., OTTONELLO S., BALDRIAN P., SPATAFORA J. W., HENRISSAT B., NAGY L.G., AURY J. M., WINCKER P., GRIGORIEV I. V., BONFANTE P. & MARTIN F. M. 2018a. — Pezizomycetes genomes reveal the molecular basis of ectomycorrhizal truffle lifestyle. *Nature Ecology and Evolution* 2: 1956-1965.

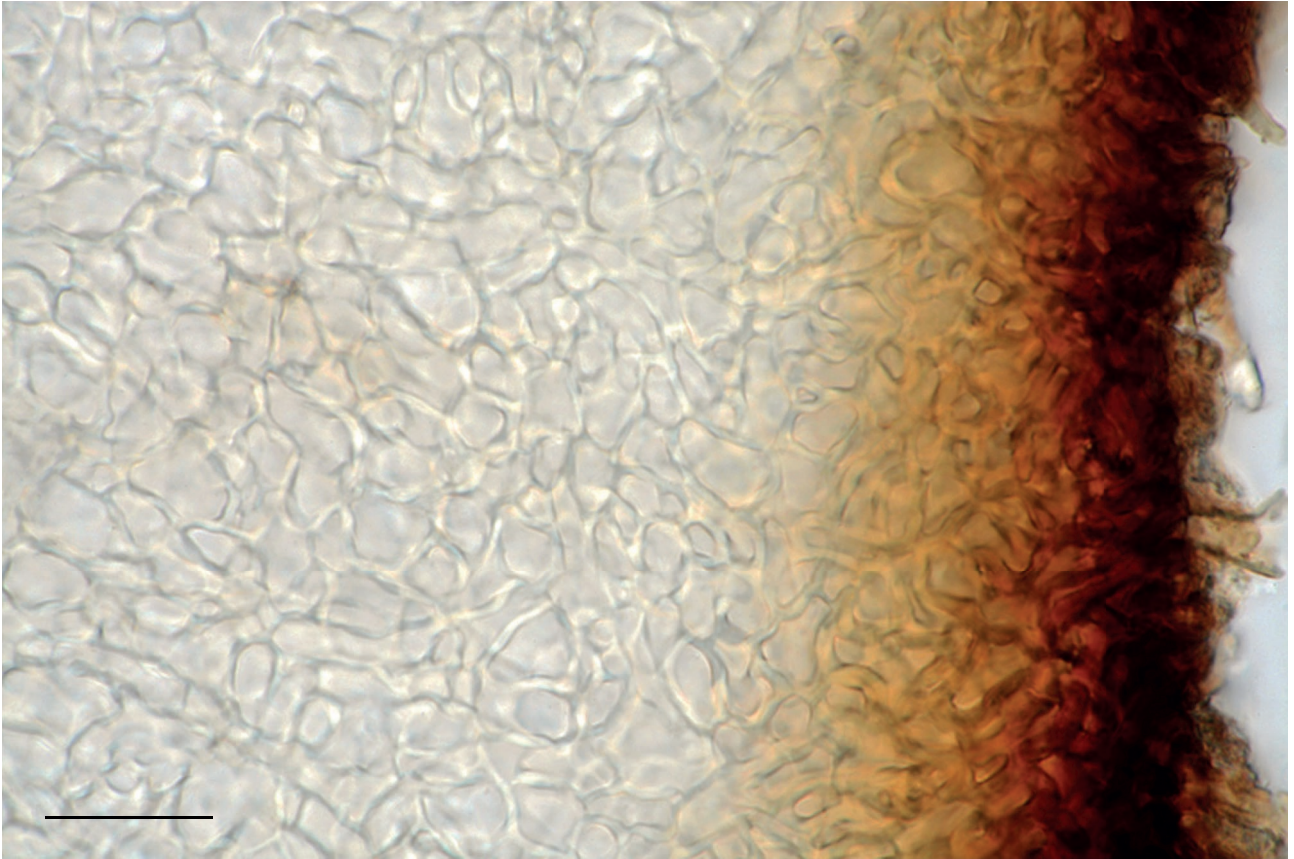
- <https://doi.org/10.1038/s41559-018-0710-4>.
- MURAT C., KUO A., BARRY K. W., CLUM A., DOCKTER R. B., FAUCHERY L., IOTTI M., KOHLER A., LABUTTI K., LINDQUIST E. A., LIPZEN A., MORIN E., WANG M., GRIGORIEV I. V., ZAMBONELLI A. & MARTIN F. M. 2018b. — Draft genome sequence of *Tuber borchii* Vittad., a whitish edible truffle. *Genome Announc.* 6: e00537–18. <https://doi.org/10.1128/genomeA.00537-18>
- PACIONI G., RAPINO C., ZARIVI O., FALCONI A., LEONARDI M., BATTISTA N., COLAFARINA S., SERGI M., BONFIGLI A., MIRANDA M., BARSACCHI D. & MACCARRONE M. 2015. — Truffles contain endocannabinoid metabolic enzymes and anandamide. *Phytochemistry* 110: 104–110. <https://doi.org/10.1016/j.phytochem.2014.11.012>.
- PAOLOCCI F., RUBINI A., RICCIONI C., TOPINI F. & ARCIONI S. 2004. — *Tuber aestivum* and *Tuber uncinatum*: two morphotypes or two species? *FEMS Microbiology Letters* 235: 109–115. <https://doi.org/10.1111/j.1574-6968.2004.tb09574.x>
- PERSOON C. H. 1801. — *Synopsis methodica Fungorum*. H. Dietrich: Göttingen.
- PICCO V. 1788. — *Melethematata Inauguralia*. Ioan. Mich. Briolus: Turin, 283 p.
- RICCIONI C., BELFIORI B., RUBINI A., PASSERI V., ARCIONI S. & PAOLOCCI F. 2008. — *Tuber melanosporum* outcrosses: analysis of the genetic diversity within and among its natural populations under this new scenario. *New Phytologist* 180: 466–478. <https://doi.org/10.1111/j.1469-8137.2008.02560.x>
- ROUX C., SÉJALON DELMAS N., MARTINS M., PARGUEY LEDUC A., DARGENT R. & BÉCARD G. 1999. — Phylogenetic relationships between European and Chinese truffles based on parsimony and distance analysis of ITS sequences. *FEMS Microbiology Letters* 180: 147–155. <https://doi.org/10.1111/j.1574-6968.1999.tb08789.x>
- RUBINI A., PAOLOCCI F., GRANETTI B. & ARCIONI S. 1998. — Single step molecular characterization of morphologically similar black truffle species. *FEMS Microbiology Letters* 164: 7–12. <https://doi.org/10.1111/j.1574-6968.1998.tb13060.x>
- SACCARDO P. A. 1888. — *Sylloge Fungorum omnium hucusque cognitorum*. Vol. VII (I). Typ. Seminarii, Padua. <https://doi.org/10.5962/bhl.title.5371>
- SCHIEBOLD J. M.-I., BIDARTONDO M. I., KARASCH P., GRAVENDI B. & GEBAUER G. 2017. — You are what you get from your fungi: nitrogen stable isotope patterns in *Epipactis* species. *Annals of Botany* 119: 1085–1095. <https://doi.org/10.1093/aob/mcw265>
- SILVESTRO D. & MICHALAK I. 2012. — raxmlGUI: a graphical front-end for RAxML. *Organisms Diversity & Evolution* 12: 335–337. <https://doi.org/10.1007/s13127-011-0056-0>
- STOBBE U., BÜNTGEN U., SPROLL L., TEGEL W., EGLI S. & FINKA S. 2012. — Spatial distribution and ecological variation of re-discovered German truffle habitats. *Fungal Ecology* 5: 591–599. <https://doi.org/10.1016/j.funeco.2012.02.001>
- TEDERSOO L., HANSEN K., PERRY B. A. & KJØLLER R. 2006. — Molecular and morphological diversity of pezizalean ectomycorrhiza. *New Phytologist* 170: 581–596. <https://doi.org/10.1111/j.1469-8137.2006.01678.x>
- TULASNE L.-R. & TULASNE C. 1851. — *Fungi hypogaei: Histoire et monographie des champignons hypogés*. F. Klincksieck, Paris.
- TURLAND N. J., WIERSEMA J. H., BARRIE F. R., GREUTER W., HAWKSWORTH D. L., HERENDEEN P. S., KNAPP S., KUSBER W. H., LI D. Z., MARHOLD K., MAY T. W., MCNEILL J., MONRO A. M., PRADO J., PRICE M. J. & SMITH G. F. (eds) 2018. — *International Code of Nomenclature for algae, fungi, and plants* (Shenzhen Code) adopted by the 19<sup>th</sup> International Botanical Congress Shenzhen, China, July 2017. Regnum Vegetabile 159. Glashütten, Koeltz Botanical Books. <https://doi.org/10.12705/Code.2018>
- VITTADINI C. 1831. — *Monographia tuberacearum*. F. Rusconi, Milan, 88 p.
- WANG Y., TAN Z. M., ZHANG D. C., MURAT C., JEANDROZ S. & LE TACON F. 2006a. — Phylogenetic relationships between *Tuber pseudoexcavatum*, a Chinese truffle, and other *Tuber* species based on parsimony and distance analysis of four different gene sequences. *FEMS Microbiology Letters* 259: 269–281. <https://doi.org/10.1111/j.1574-6968.2006.00283.x>
- WANG Y., TAN Z. M., ZHANG D. C., MURAT C., JEANDROZ S. & LE TACON F. 2006b. — Phylogenetic and population study of the *Tuber indicum* complex. *Mycological Research* 110: 1034–1045. <https://doi.org/10.1016/j.mycres.2006.06.013>
- WHITE T., BRUNS T., LEE S. & TAYLOR J. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. *PCR Protocols: A Guide to Methods and Applications* 18: 315–32.
- ZAMBONELLI A., IOTTI M. & MURAT C. (EDS) 2016. — True truffle (*Tuber* spp.) in the world. *Soil Biology* 47. Cham, Switzerland: Springer International Publishing, 436 p. <https://doi.org/10.1007/978-3-319-31436-5>
- ZARIVI O., CESARE P., RAGNELLI A.M., AIMOLA P., LEONARDI M., BONFIGLI A., COLAFARINA S., POMA A.M., MIRANDA M. & PACIONI G. 2014. — Validation of reference genes for quantitative real-time PCR during *Tuber melanosporum* developmental stages. *Phytochemistry* 116: 78–86. <https://doi.org/10.1016/j.phytochem.2015.02.024>

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APPENDICES



APPENDIX 1. — Surface of an ascoma of *Tuber aestivum* Vittad. showing transverse streaks in peridium warts.



APPENDIX 2. — *Tuber melanosporum* Vittad. epitype: different cut of peridium structure  $\times 40$ . Scale bar: 50  $\mu\text{m}$ .

## C A R I N T H I A C Æ.

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cendō aquose brunescat, ac denique terreus fiat, apice in foramellum inæqualiter lacerum dehiscendo; polline feminali non rubro, sed lilacei omnino coloris.

## CXXXIII.

## LYCOPERDON ÆSTIVUM.

*Lycoperdon subglobosum* solidum glabrum, subterraneum, radice destitutum. Tuber æstivum, pulpa subobscura, minus lapida, minusque odora. *Michel. N. Gen. plant. p. 221. N. 2.*

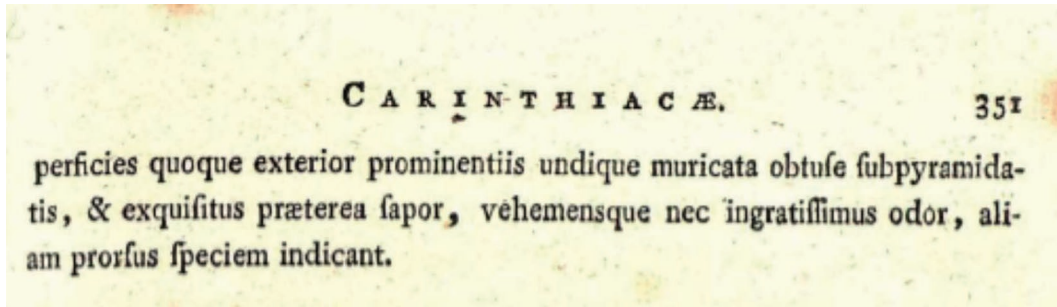
*Lycoperdon subglobosum* subterraneum solidum & scabrum, basi & radice carens, capsulis feminalibus magnis. *Gleditsch. Meth. fung. p. 157. N. 8.* Tuberum tertium genus in Ananiensi, & Tridentino tractu proveniens, lævi cortice, colore subrufo, cæteris longe minus, insipidum, & gustu injucundo. *Matthiol. in Dioscor. p. 271.*

Multus de Tuberibus, quæ *Tartuffeln* vulgo vocitant, per Carinthiam, Carnioliamque omnem ubivis sermo. Rudis plebecula, si eandem desuper percontari libeat, plurima, quæ narret, habet. Mira sunt, quæ de eorumdem virtute, præstantia, exquisito sapore, odore aromatico, difficultate habendi, tum pretio &c. passim ventilantur. Et tamen in omnibus narrationibus istis, rei quidem subest nihil. Tubera certe nostra, quotquot vidi (& vidi sane plurima; quia magna in copia undique adferuntur quotannis dividenda) ab iis Hispaniarum, & Hetruriæ, quæ itidem probe novi, plurimum differunt. Copiosissime apud nos eruuntur Mensibus Majo, Junio, Julio & Augusto *im Gleinacherwald, und Resnick bey Ferlach*; tum in collibus sylvosis abiegnis *bey Krastowitz und Freudenberg*; nec non in celebri monte *Loibl,*

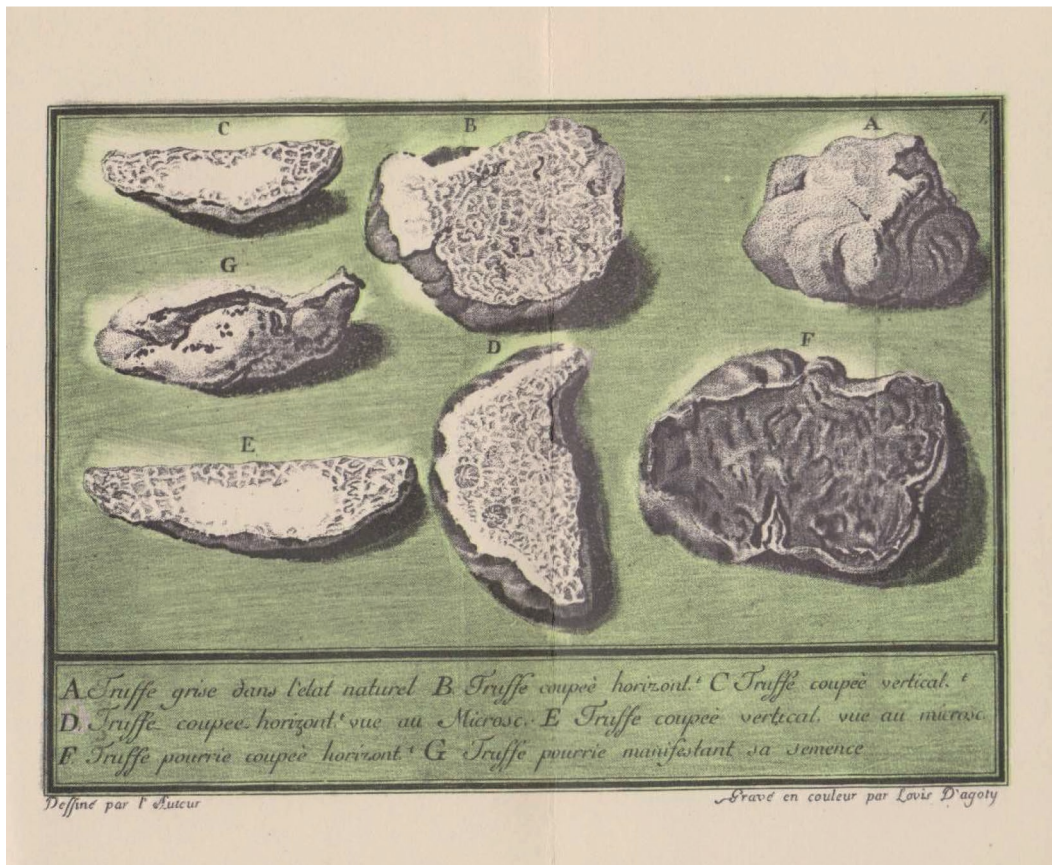
X x 2

&amp;c.

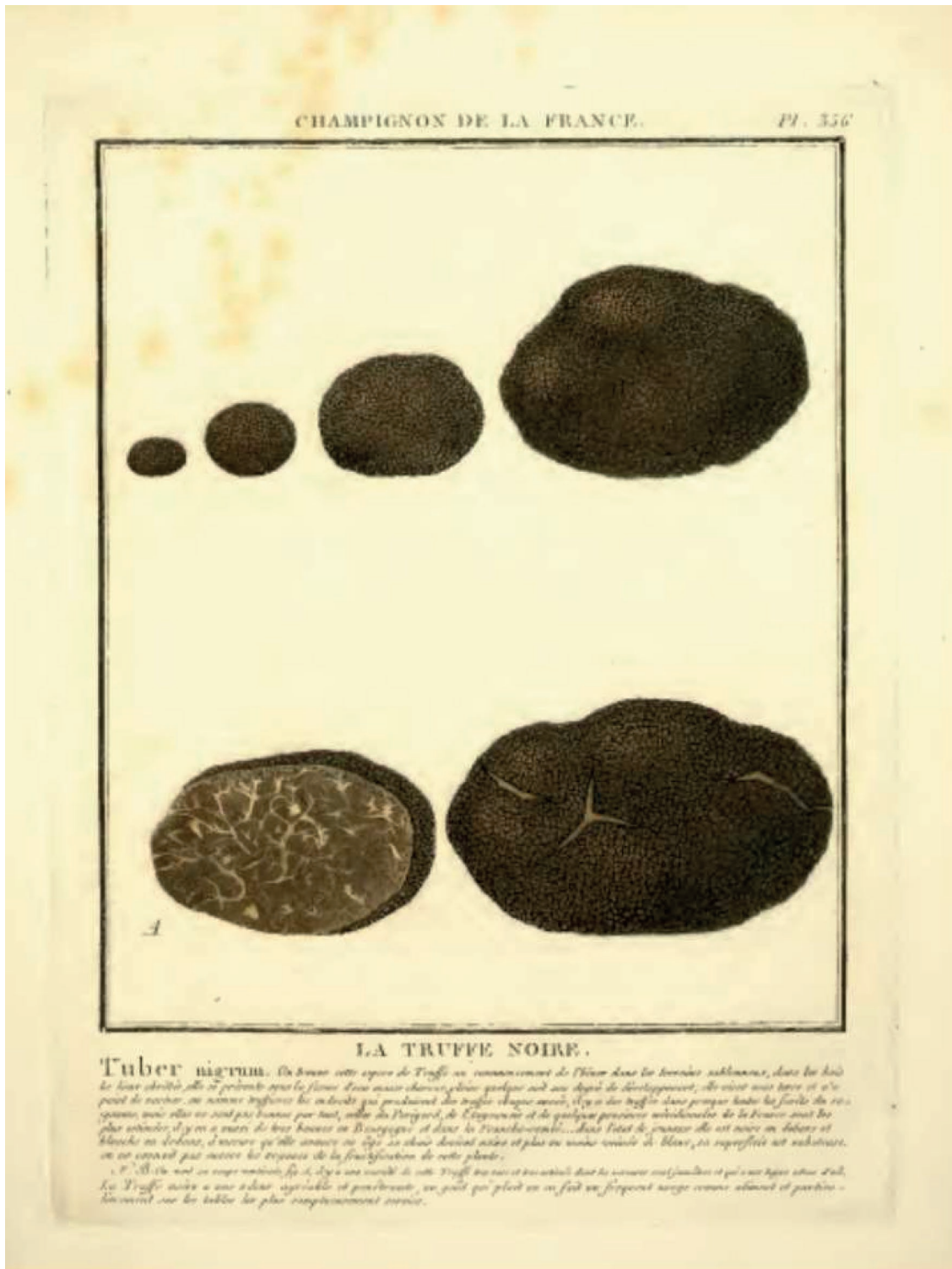
&c. Neque canibus, hic quidem locorum, ad eadem detegenda, opus est. Ipse ego a peritis in loca deductus ista, de terra evulsi; quamvis ex ea protuberare viderem nihil; at monuerunt illi, digitoque, quo quodvis conssepul-tum esset loco, indicarunt. Scilicet ista terræ merfa sunt tota, ut de iis ex-flet nihil; extima duntaxat supremi segmenti superficies, in eadem cum terra libella, ab eadem denudata est, ut adeo, quoniam plerumque vix, aut ne vix quidem colore extus, a terra ipsa differre solent, non nisi ab adfueto jam discernantur oculo; canibus inutiliter quærenda, quæ odoris sint expertia. Hæc nisi propria didicissem experientia, haud assererem. Magnitudo illis nucis castaneæ, aut drupæ Juglandis; corpus subrotundum, inordinate sphæ-roïdicum, solidum; extus fordide albidum, & ex albido obsolete subrubes-cens, instar pomorum terræ (solanum tuberosum L.), dein dilute fuscescens, nigricans denique; glabrum id semper inveni; etsi cuticula extima, eaque te-nerrima, hinc inde disrupta, & exstans, squamulas exhiberet fursuraceas, quibus tamen nec asperum, nec scabrum, minus muricatum redderetur exte-riore in superficie. Totum sub terra occultitur; præter superficiem cuticulæ supremam a terra denudatam. Radices nullæ; capillares duntaxat, brevissi-mæque in quibusdam fibrillæ; æque paucissimæ. Caro intus spongiosa, sub-coriacea, primum alba, tum obsolete, & triste ex albido subcinerascens, ma-turitate? aut exsiccatione? fuscescens, subnigricans, ac tum etiam cellulolo-porosa. Odor recentium nullus, aut certe debilissimus & subnauseosus. Sa-por nullus, nisi imaginarius. Expetuntur tamen a multis ad epulas, & pre-tio emuntur, quia Tubera, vulgo *Tartuseln* sunt. Gustavi recentia, mandu-cavi etiam in disculos secta orbiculares, desiccata, & variis cocta in jusculis; nil tamen saporis, minus deliciarum in hoc fatuo cibo detegere potui genere. Plurimum itaque a brumalibus Michellii Tuberibus (Lycoperd. Tubere. L.) di-versa; quorum non moles solum major, aterrimusque corticis color, sed su-per-



APPENDIX 3. — Continuation.



APPENDIX 4. — Lectotype of *Tuber griseum* Borch ex Pers. sanct. Fries (1823), "Truffe grise" De Borch (1780 1st figure).



APPENDIX 5.— Lectotype of *Tuber nigrum* Bull. (*Herbier de la France* 8: t. 356, 1788).