

***Tuber asa* and *T. gennadii*. A close morphological study of two species often confused in the past with a brief historical bibliographic summary**

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Summary: The authors describe *Tuber asa* and *T. gennadii*, showing the differences between the two species. A historical overview that highlights how the species have been often confused in the past is also given. The study is accompanied by images in their habitat and by microscopical photographs, drawings and original plates.

Keywords: Ascomycota, *Tuberaceae*, hypogeous Pezizales, taxonomy, Greece, Italy.

Résumé : les auteurs décrivent *Tuber asa* et *T. gennadii*, montrant les différences entre les deux espèces. Un aperçu historique qui met en lumière comment les espèces ont été confondues dans le passé est également donné. L'étude est accompagnée par des images dans leur habitat et par des photographies microscopiques, des dessins et des planches originales.

Mots-clés : Ascomycota, *Tuberaceae*, Pezizales hypogés, taxinomie, Grèce, Italie.

Introduction

The two species treated in this paper share similar habitats while remaining morphologically very different. The habitat of the collections is Mediterranean woods at low altitudes or even directly by the sea and even on sandy soil in various mixtures of *Pinus halepensis*, *P. pinea*, *Quercus coccifera*, *Juniperus oxycedrus* as well as various species of *Cistaceae* in broad opening. Greek collections evidence that *T.gennadii* and *T. asa* co-exist and, in one case, within a few centimeters; we also found *Picoa* sp. and *Terfezia* sp. in the same habitats. MONTECCHI *et al.* (2001) published a similar study. Our discussion adds important data for both *Tuber* species and integrates information published on this subject since 2001. The decision to list the chronological history of the two species together may seem chaotic, but we decide to do so because over past years these two species have been confused. Part of this confusion arises because some past mycologists disregarded the holotypes that set the standard for morphology characterizing the species.

Materials and methods

Morphological characters are described from fresh specimens. Microscopic characters were studied from sections mounted in water to observe pigments and to measure structures. Melzer's reagent and cotton blue were used to

highlight the ornamentation and Congo red for the walls of cells. Spores dimensions are based on at least 60 randomly selected spores, excluding ornamentation. Our descriptions are conform to the holotypes of *Tuber asa* and *T. gennadii* at the Muséum national d'histoire naturelle, Paris (PC), as described by J. Trappe (personal communication).

Taxonomy

Tuber asa Tul. & C. Tul., *Fungi Hypogaei*: 149 (1851).

≡ *Tuber asa-foetida* Lespialt, *manuscriptum in schedis propris*.

Original diagnosis

Fungus globosus vulgo nucis magnitudine, undique irregulariter gibboso-sulcatus, sordideque luteolus. Peridium leve, tenue, glabrae valde haerens. Caro firma griseo-lutea venis labyrinthis albidis vel griseis, anastomosantibus, nec non lineis angustissimis raris nigrescentibus marmorata. Sporangia ovato-rotundata, sporis globosis (et quidem sphaericis ac Omm.032-042 diametro mentientibus) elegantissime reticulatis (maculis saepius hexagonis) maturitatisque tempore saturate luteo-fuscis seu rufulis foeta. Odor gravissimus illius Asae foetidae admodum aemulus, penitus intra paucos dies evanescit. (Lespiald.) Raro effoditur in sabuletis prope Neracum, mense januario. (Lespiald.) Specimina sicca sectaque vidimus quae Balsamiam vulgorem quodammodo mentiuntur.



Fig. 1 - *Tuber asa*. Collection 2008 from Schinias (Greece) – photo: V. Kaounas.



Fig. 2 - *Tuber asa*. Immature collection under *C. monspeliensis* (S.Pancrazio S.no-Italy) – photo: C. Agnello.

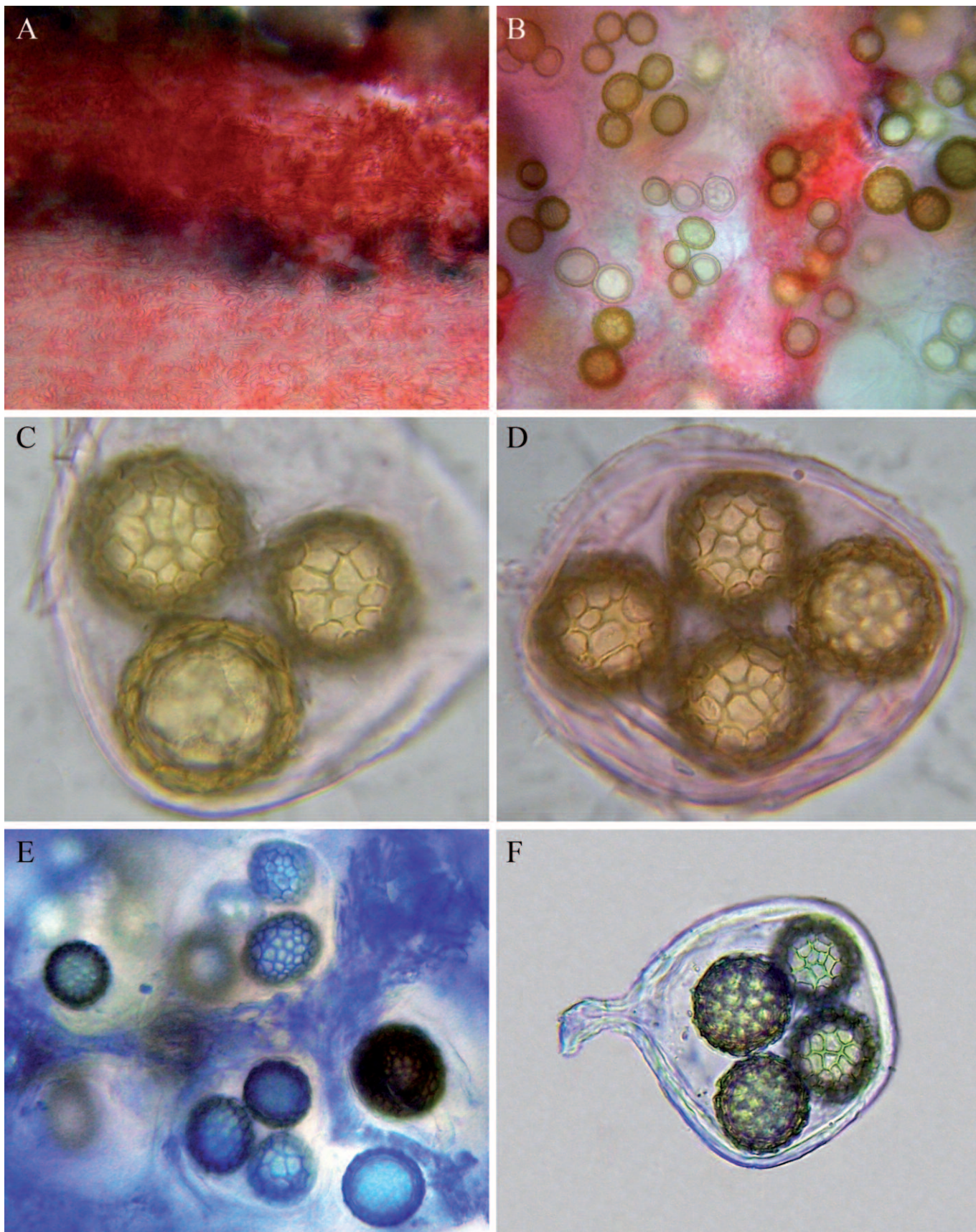


Fig. 3 – *Tuber asa* (photos: C. Agnello)
 A. Peridium in Congo red.
 B-C-D. Asci and spores in Congo red.
 E-F. Asci and spores in cotton blue.

Description

Ascomata irregularly globose, tuberiform, 2–5 (7) cm broad, nearly smooth, ochraceous yellowish with reddish brown spots. **Peridium** entirely by parallel or interwoven hyphae, up to 5–6 μm broad. **Gleba** yellowish grey to beige with whitish veins. **Odor** generally unpleasant; of stale, rotten wood but sometimes pleasant.

Asci randomly embedded in the fertile tissue, subglobose to broadly ellipsoid, hyaline, with or without a short stem, 70–95 μm broad, 1–4 (5) spored. **Spores** globose or broadly ellipsoid, 25–45 \times 22–40 μm , $Q < 1.2$, yellowish brown at maturity; ornamentation is an alveolate reticulum of polygonal meshes, the ridges 1–3 (5) μm tall.

Season, distribution and habitat

Hypogeous or emergent, autumn to spring in Mediterranean ecosystems at low elevations, in dry and sandy places, in deserts and beaches, under *Cistus incanus*, *Cistus salvifolius*, *Tuberaria* sp., *Helianthemum* sp., *Ephedra* sp., among *P. halepensis*, *Juniperus oxicedrus*, etc.

Collections examined (partial)

GREECE: 09.05.2008, Schinias (Attika) alt. 5 m; 03.01.2011, Rafina (Attika), alt. 60 m; 22.02.2011, Nea Makri (Attika) alt. 50 m. Vasileios Kaounas collected and identified all Greek specimens which are conserved in his personal herbarium. ITALY: 17.04.2008, Ginoso Marina (Apulia) at sea

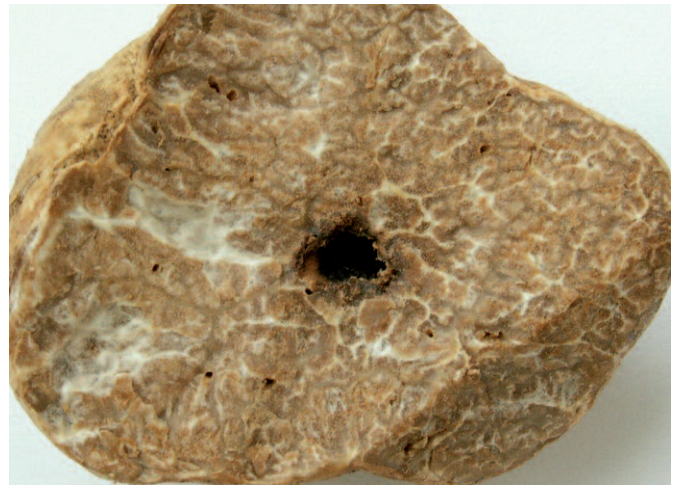
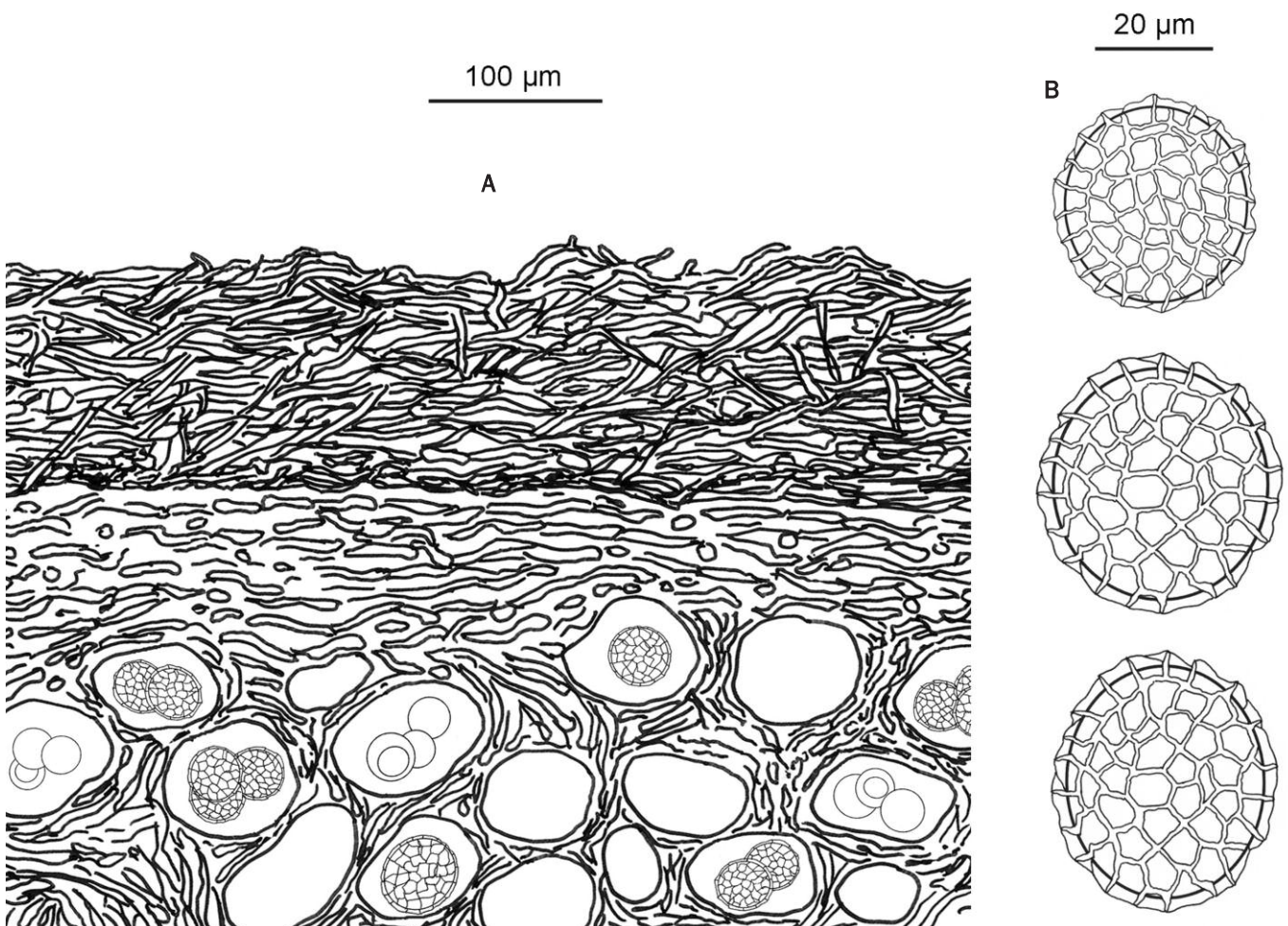


Fig. 2 – *Tuber asa*. Particular of gleba; collection 2008 from Ginoso Marina (Italy) – photo: C. Agnello.

level, leg. Emanuele Pepe, det. C. Agnello; 08.12.2008, Canole (Apulia), alt. 30 m, leg. Eugenio Murciano, det. E. Murciano & C. Agnello; 20.02.2009, S. Pancrazio S.no (Apulia), alt. 50 m, leg. and det. Adolfo Delle Donne & C. Agnello; 11.04.2009, Manduria (Apulia), alt. 100 m, leg. Lucio Maraschio, det. C. Agnello. All Italian collections are conserved in C. A. personal herbarium.



Tab. 1 – *Tuber asa*. A. Peridium and part of gleba. B. Spores – Drawing: C. Agnello.

Notes

This species can be confused with *T. oligospermum* (Tul. & C. Tul.) Trappe because it often shares the same habitat. *T. oligospermum* differs microscopically from *T. asa* by its totally globose spores (that's why the species was originally described as *Terfezia oligosperma* Tul. & C. Tul.), asci with or without short-stalks, peridium with hyphae interwoven so that the section can appear to consist of elongated hyphal cells mixed with small sphaerical cells, but in reality those are cross sections of elongated hyphae. *T. asa* fruits in October or November to early June. In our experience, *T. asa* contrasts with many other *Tuber* species in that immature specimens rarely mature when stored in a refrigerator.

Tuber gennadii (Chatin) Pat., *Bull. Soc. mycol. Fr.*, 19: 255 (1903)

- ≡ *Terfezia gennadii* Chatin, *Bull. Soc. bot. Fr.*, 43: 611 (1896).
- ≡ *Tuber lacunosum* Mattir., *Malpighia*, 14: 53 (1900).
- ≡ *Delastreopsis gennadii* (Chatin) Reichert, *Palestine J. Botany*, 4: 198 (1944).
- ≡ *Loculotuber gennadii* (Chatin) Trappe, Parlade & I.F. Alvarez, *Mycologia*, 84(6): 927 (1992).

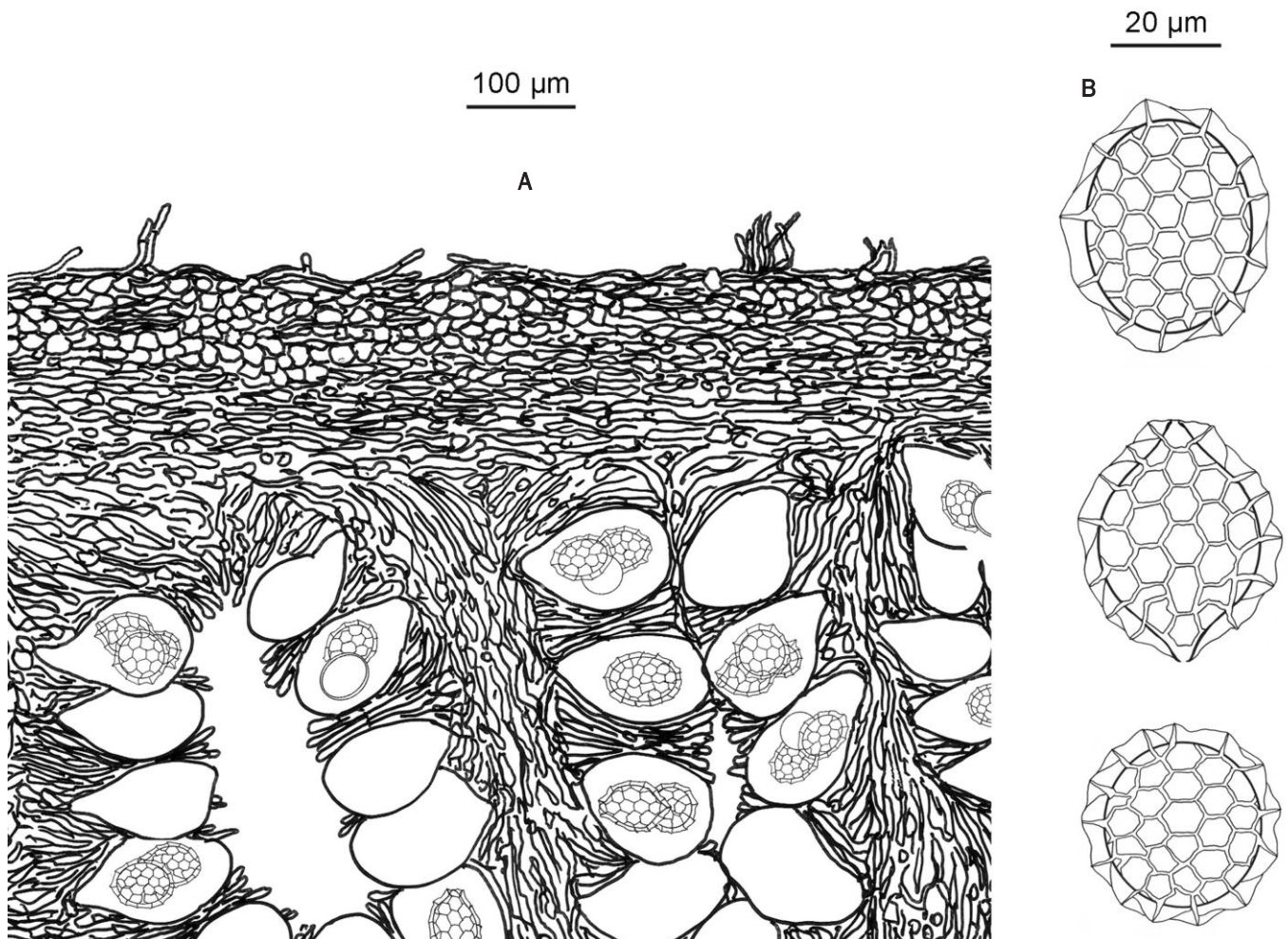
Original diagnosis

Troisième sorte. Quiza. Tubercules fort petits, du poids de 8 à 15 grammes. Périderme jaunâtre. Chair d'un gris brun assez foncé, ferme, avec d'assez fines marbrures blanches, odeur et saveur peu sensibles, nullement alliées. Sporangés allongés (rappelant ceux de *Tuber magnatum* ou Truffe à l'ail de Piemont), bispores, rarement trispores; spores rondes, du diamètre de 45 μ à 51 μ , parfois ellipsoïdes, comme dans les *Tuber* et *Tirmania*, avec des diamètres de 36 μ sur 46 μ .

Description

Ascomata irregularly globose, tuberiform, 1–4 cm broad, smooth or pubescent in places, greyish yellow with ochraceous brown spots, often with attached grains of sand. **Peridium** suprapellis a discontinuous pubescence of septate yellowish hyphal tips 4–5 (7) μ broad; pellis a discontinuous pseudoparenchyma of *textura angularis* with cells up to 15 μ broad; subpellis of filamentous hyphae 5–7 (10) μ broad. **Gleba** initially greyish, later dark brown, with small but visible locules 0.2 to 1 mm broad, separated by thin whitish veins. **Odor** unpleasant, resembling overripe cheese or naphthalene.

Asci produced in a hymenium, clavate to ellipsoid, with a long-stemmed, 100–150 \times 50–90 μ m, hyaline, 1–3 (4) spored. **Paraphyses** septate, shorter or longer than the asci, with enlarged apex up to 3–6 (8) μ m. **Spores** yellowish brown at maturity, globose, broadly ellipsoid or citriform (eye-shaped), 32–42 (55) \times 25–32 (35) μ m, ornamented by ridges 3–5



Tab. 2 – *Tuber gennadii*. A. Peridium and part of gleba. B. Spores – Drawing: C. Agnello.



Fig. 5 – *Tuber gennadii*. Collection of mature specimens (Schinias, Greece) – photo: V. Kaounas.



Fig. 6 – *Tuber gennadii*. Immature samples – photo: V. Kaounas.

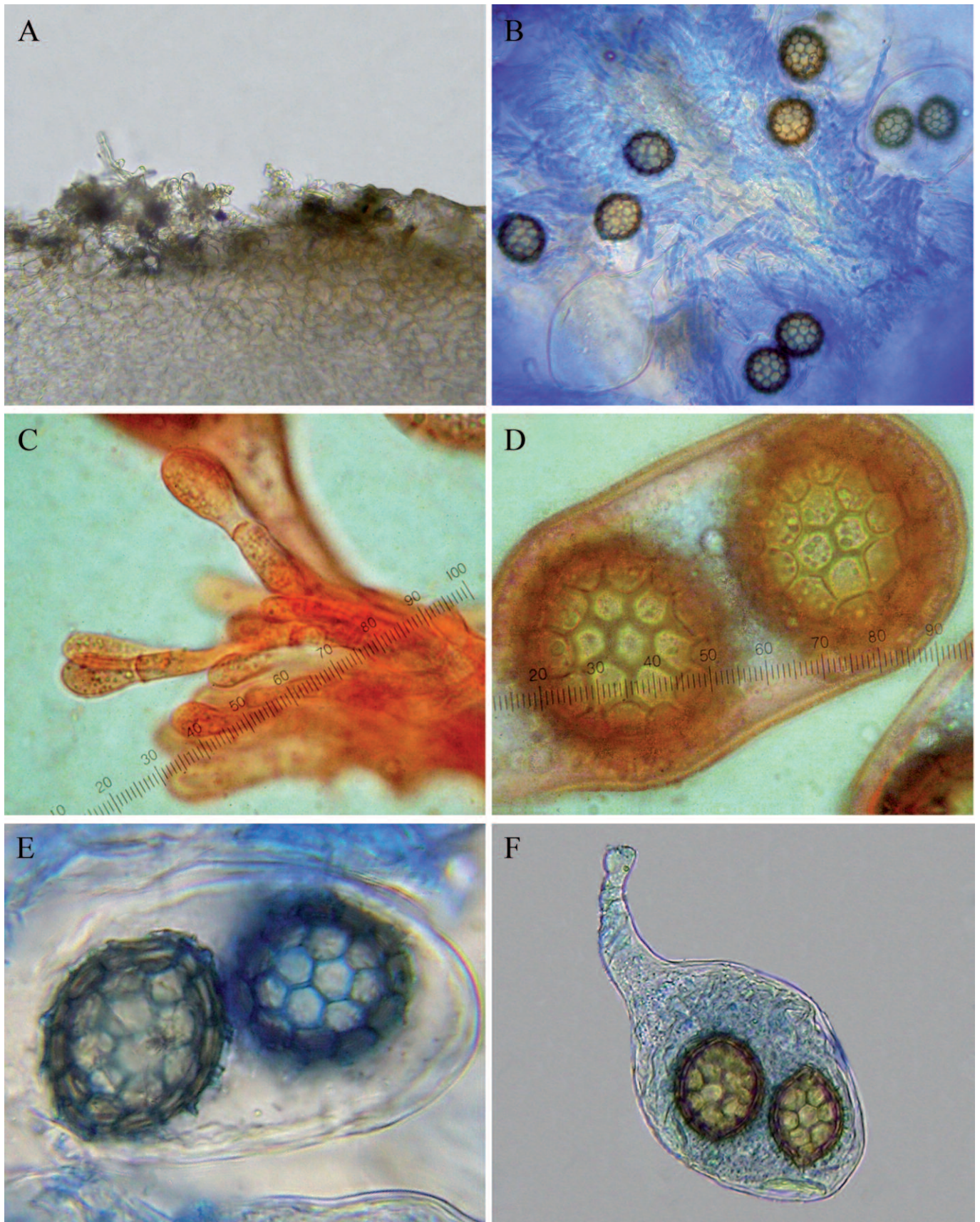


Fig. 7 – *Tuber gennadii* (photos: C. Agnello, except C & D: D. Sofronis)
 A. Peridium in water. B. Asci and spore in cotton blue; peridium in Congo red.
 C. Paraphyses in Congo red. D. Spores in Congo red.
 E-F. Ascus and spores in cotton blue.

(7) µm tall which form a hexagonal reticulum. Focusing in some spores is possible to see a smallest double reticulum.

Season, distribution and habitat

Occurs in late autumn to spring, in groups, in underground, in Mediterranean ecosystems at low elevations in dry and sandy places, on beaches, along with various *Cistaceae* (*Cistus* sp., *Tuberaria* sp., *Helianthemum* sp.) among *Pinus pinea*, *P. halepensis* and *Quercus coccifera*.

Collections examined

GREECE: 08.04.2009, 24.04.2009, 12.04.2011, Schinias (Attika), alt. 5 m. Vasileios Kaounas collected and identified all Greek specimens which are conserved in his personal herbarium.

Note

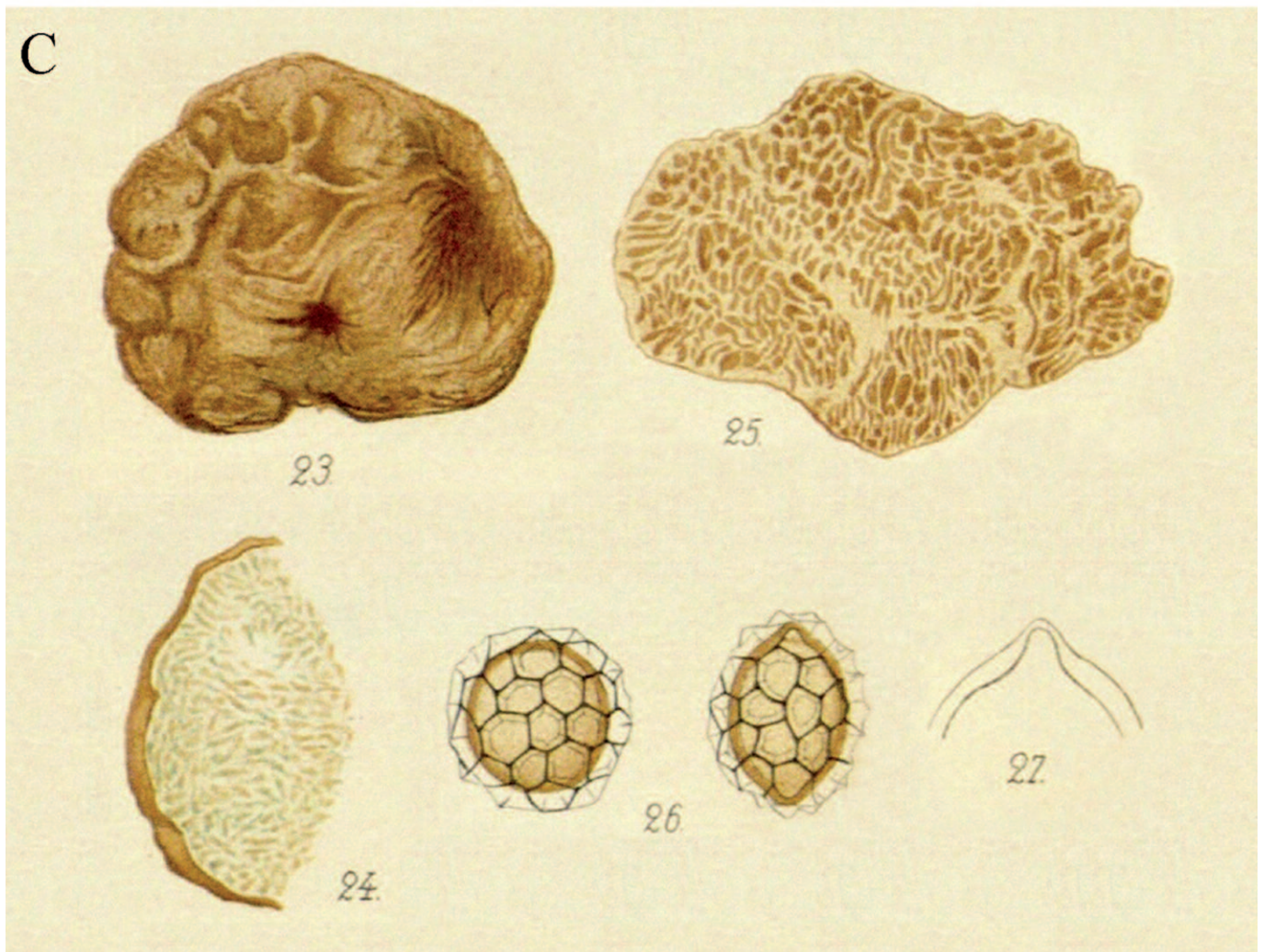
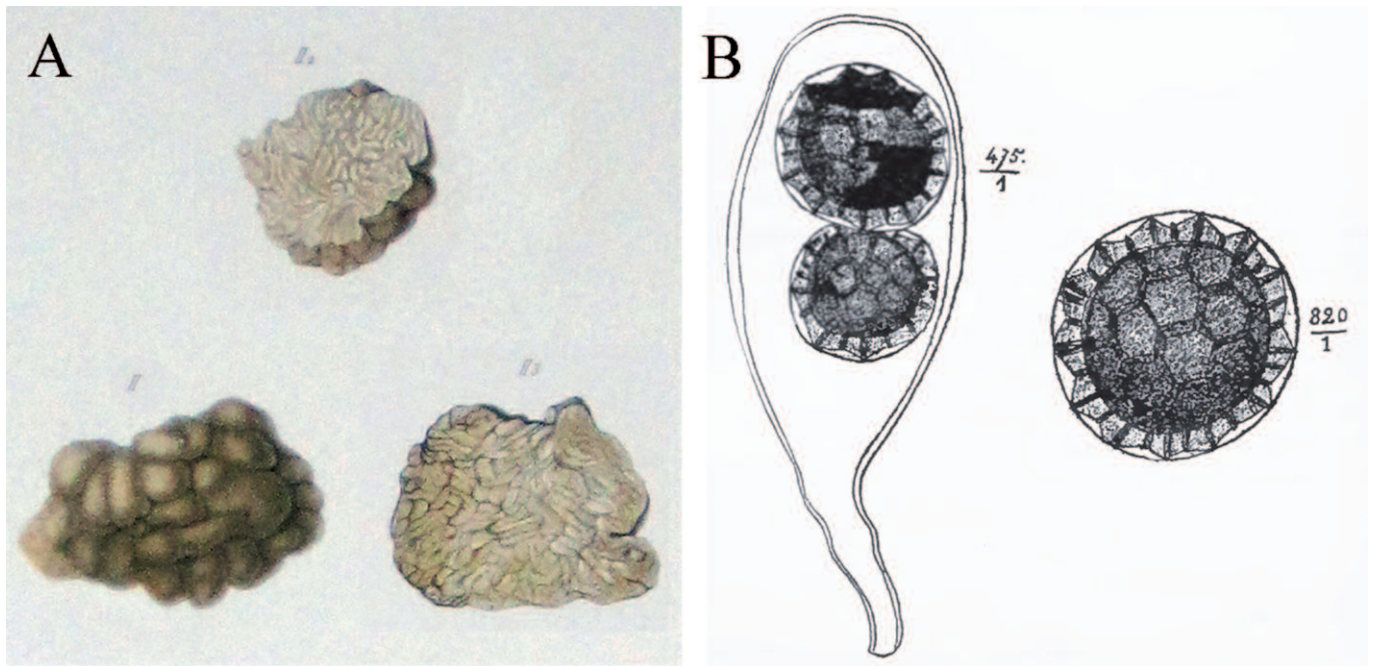
Macroscopically, the mature specimens of *T. gennadii* can not be confused with any other *Tuber* species because its gleba is crossed by locules. This is the reason why the new genus *Loculotuber* was published by ALVAREZ *et al.* (1992). Microscopically, the main distinctive characters are the citriform, eye-shaped spores (about 30%) and the particular arrangement of asci around the hollow parts.



Fig. 8 – *Tuber gennadii*. Particular of gleba; collection 2011 from Schinias (Greece) – photo: V. Kaounas.

Chronology

- TULASNE & TULASNE (1851) describe *Tuber asa*, a species previously treated in a handwritten paper by Lespiault as “*Tuber asa-foetida*”.
- FERRY DE LA BELLONE (1888) claims to have been able to study twice *Tuber asa* and gives a faithful description.
- CHATIN (1896) describes *Terfezia gennadii* based on gatherings made in the region of the Peloponnese (GR) and sent by the Greek plant pathologist Panagiotis Gennadius (the name of the species is dedicated to him).
- SACCARDO & SYDOW (1899), in *Sylloge Fungorum* XIV, translate from French into Latin the original diagnosis of *Terfezia gennadii* Chatin.
- MATTIROLO (1900) publishes, after Sardinian and Sicilian collections, *Tuber lacunosum* sp. nov. (“*Tuber lacunosus*”), adding to the extensive description a beautiful plate made by Lionello Petri.
- PATOULLARD (1903), after Algerian collections, provides the new combination *Tuber gennadii* (Chatin) Pat.
- MATTIROLO (1905) disputes the placement in the *Terfezia* genus by Chatin but has no doubt to admit that *Tuber lacunosum* and *Tuber gennadii* are conspecific. In the same time, it is strange that, in his next paper from Portugal (MATTIROLO, 1906), he maintains *Tuber lacunosum*.
- BATAILLE (1921) lists three species: *Terfezia gennadii*, *Tuber asa*, *Tuber lacunosum*. This suggests that the author ignored the work of PATOULLARD (1903) and the synonymy proposed by MATTIROLO (1905). This is confirmed by the bibliographic list which does not report the contributions of these authors.
- REICHERT (1944) publishes the combination in *Delastreopsis gennadii* (Chatin) Reichert. Our records indicate that this systematic position will not result in subsequent authors.
- CERUTI (1960) places in synonymy *Tuber asa* and *Tuber gennadii*, claiming to have been able to study one Mattirollo’ specimen of *Tuber lacunosum* and one Tulasne’ specimen of *Tuber asa* and did not find any difference.
- MALENÇON (1973), describing hypogeous fungi from Africa, considers *Tuber asa* and *Tuber gennadii* as synonyms. The species described in this publication, in our opinion, has to be referred to *Tuber gennadii*.
- MORENO *et al.* (1991): the species treated in this study appears to be *Tuber gennadii* and not *Tuber asa*.
- ALVAREZ *et al.* (1992): after the type study of *Tuber gennadii*, they clearly separate the two species and recombine it in the new genus *Loculotuber*. We believe this study is a key moment in the history of the two species because it becomes the start-point to stimulate the further investigation.
- ASTIER (1998) considers *Tuber lacunosum* Mattirollo as a synonym of *Tuber asa* and does not treat *Tuber gennadii*. The French author does not give any information that led to this choice. The species illustrated is, in our opinion, unequivocally *Tuber gennadii*.
- MONTECCHI & SARASINI (2000) support the strict separation of the two species because, since 1992, they devoted many years of research in the original location of the type in order to find and to study material and also make morphological comparison. The two authors consciously choose to not adopt the new genus *Loculotuber*, keeping the taxon *Tuber gennadii*.
- MONTECCHI *et al.* (2001) give an account of their researches and develop a chronology as mentioned in “Introduction”.
- RIOUSSET *et al.* (2001) present *Tuber asa* placing it in synonymy with *Tuber lacunosum* Mattirollo, again ignoring *Tuber gennadii* (Chatin) Pat.
- CERUTI *et al.* (2003) accept the combination *Loculotuber gennadii* and treat *Tuber asa* as a distinct species.
- GORI (2005; 2010) has good knowledge of the species and its peculiarities feels the need to maintain both species in the genus *Tuber*.
- BONITO *et al.* (2010a; 2010b) sequenced collections from Spain and Italy of *Tuber gennadii* and conclude its placement in genus *Tuber*.



Tab. 3 – Original plates that accompanied the diagnosis (adapted table)

A. *Tuber asa* in TULASNE & TULASNE, 1851, tab. V, fig. II, II2, II3.

B. *Terfezia gennadii* in CHATIN, 1896, p. 616.

C. *Tuber lacunosum*, drawing of Lionello Petri, in MATTIROLO, 1900, tab. 1, fig. 23, 24, 25, 26 and 27.

Conclusions

Tuber asa and *T. gennadii* are two species morphologically very different, beyond the systematic position: this was clear to the old authors. Until recently, some authors have made mistakes in synonymy or determination. We believe that certain confusions started with CERUTI (1960) but it is not clear why after ALVAREZ *et al.* (1992) several authors did not discuss about these species separately.

Acknowledgements

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