

Preliminary notes on the genus *Tarzetta* (Pezizales) with typifications of some species and description of six new species

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Abstract: Species of *Tarzetta* are morphologically well-defined but their taxonomy is confused because of the multiple interpretations of the oldest names, i.e. *T. catinus* and *T. cupularis*. The exploration of this genus with molecular and ecological data gave us the opportunity to partially clarify its taxonomy and to propose typifications of names. The type collections of *T. pusilla*, *T. gaillardiana*, and *T. spurcata* in the sense of Harmaja were reviewed. *Hypotarzetta insignis*, a semi-hypogeous species, is reinstated in *Tarzetta* based on molecular data. New species are also described and illustrated: *T. gregaria* sp. nov. is characterized by its small size and gregarious orangish ascocarps, *T. alnicola* sp. nov. is a small species associated with alders in riparian woodlands, *T. alpina* sp. nov. grows in boreo-alpine regions, *T. sepultarioides* sp. nov. is characterized by its *Sepultaria*-like appearance and ascus base shape, *T. melitensis* sp. nov. is a small species described from Malta, and *T. quercus-ilicis* sp. nov. is a small, sessile species, associated with *Quercus ilex* in Mediterranean regions. *T. betulicola* is a provisional name proposed for a species from the *T. catinus* complex, probably associated with birch trees.

Keywords: Ascomycota, Pyrenomataceae, Otideaceae, Tarzettaceae, rDNA analyses, nomenclature, 6 new species.

Introduction

The name *Tarzetta* was established by COOKE (1879) as a subgenus of *Peziza* Fr., but no type was designated. It was later combined to the genus *Tarzetta* by LAMBOTTE (1888) and typified by ROGERS *et al.* (1971) with *Peziza tarzetta* Cooke as type-species (according to Art. 22 ICN). This name was considered as a later synonym of *Peziza catinus* Holmsk. (NANNFELDT, 1938). Consequently, *Tarzetta* replaced *Pustulina* Eckblad, a new name published in replacement of *Pustularia* Fuckel, *nom. illeg.* (ECKBLAD, 1968).

The case of orthography of *Tarzetta* was previously discussed by VAN VOOREN (2010) where we explain why we do not agree with YAO & SPOONER (2002) who proposed to correct it as *Tazzetta*.

The most complete work on *Tarzetta* was published by HÄFFNER (1992) but the typification of *T. catinus* (Holmsk.) Korf & J. K. Rogers and *T. cupularis* (L.) Lambotte, two of the oldest names in the genus, were not treated. Serious confusion exists in the application of these names, and the precise interpretations differ in the literature. This is mainly due to the exceedingly few microscopic differences observed between these two species. In its current concept, the genus *Tarzetta* contains approximately ten species that are distributed worldwide (source: MycoBank database, accessed on Sept. 2019). Species of *Tarzetta* form ectomycorrhizae (EcM) with trees, e.g. *Fagus sylvatica* (TEDERSOO *et al.*, 2006), *Quercus douglasii* (SMITH *et al.*, 2007), *Alnus* spp. (TEDERSOO *et al.*, 2009), *Pinus monticola* (SOUTHWORTH *et al.*, 2009) and *Corylus avellana* (BENUCCI *et al.*, 2014). Our phylogenetic analyses (Fig. 1 and 2) suggest a higher diversity and a possible host-specificity for several species based on the EcM sequences available in public databases (e.g. GenBank).

The goal of this work is to clarify the taxonomy of the species mainly described and illustrated by BOUDIER (1905-1910) and HARMAJA (1974), based on type studies, morphological and phylogenetic data from the internal transcribed spacer region (ITS) and the 28S large-subunit (LSU) of the rDNA. This work will provide a new framework for future studies. A type revision of *T. gaillardiana* (Boud.) Korf & J.K. Rogers, *T. spurcata* (Pers.) Harmaja and *T. pusilla* Harmaja is also provided. Six new species are described and illustrated based on morphological, ecological, and molecular data: *Tarzetta alnicola*, *T. alpina*, *T. gregaria*, *T. melitensis*, *T. quercus-ilicis*, and *T. sepultarioides*.

Material and methods

Morphology and cytology. — Observations were made from both fresh and dried material. For dried samples, parts of the specimens were rehydrated for approximately eight hours in tap water at room temperature. Observations of microscopic characters were

made in various solutions and stains: tap water, Cotton Blue (CB) in lactophenol, 5% potassium hydroxide (KOH), and Lugol's solution (IKI) to test the amyloid reaction. Measurements were made on 30 ascospores from each collection, mounted in water, under the 100× oil immersion lens of a transmission light microscope, excluding the ornamentation. "X" represents the average value of spore dimensions, and "Q" the ratio between spore length and width. Macrophotographs were made *in situ* using digital cameras, while micrographs were taken using a microscope mounted digital camera. Line drawings were made freehand to scale. The symbol † is used to annotate observations from rehydrated material.

DNA extraction, amplification and sequencing. — DNA was extracted using the method described in VAN VOOREN *et al.* (2015). Loci that were amplified included the ITS (ITS1, 5.8S and ITS2 rDNA) and LSU (D1, D2 and D3 of the 28S rDNA). Sequences obtained during this study were deposited in GenBank under the accession numbers listed in Table 1.

Phylogenetic analyses. — BLAST® was used to select the most highly similar ITS and LSU sequences from the International Nucleotide Sequence Database Collaboration public databases. For the LSU locus (662 sites), the evolutionary analyses were conducted in Mega X (KUMAR *et al.*, 2018) with 68 nucleotide sequences. The alignment was done with the MUSCLE algorithm (EDGAR, 2004). The evolutionary history was inferred by using the Maximum Likelihood (ML) method based on the General Time reversible (GTR) model (NEI & KUMAR, 2000) with 1000 bootstrap iterations. The LSU tree with the highest log likelihood (-3494.72) is shown in Fig. 1. The percentage of trees in which the associated taxa clustered together is shown next to the branches. Initial tree(s) for the heuristic search were obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach, and then selecting the topology with superior log likelihood value. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.5999)]. The rate variation model allowed for some sites to be evolutionarily invariable ([+I], 42.22% sites). For the ITS locus (515 sites), 72 sequences were aligned in Mesquite (MADDISON & MADDISON, 2015) using the MUSCLE algorithm. A maximum likelihood (ML) search was performed using RAXML (STAMATAKIS, 2014) with the GTR GAMMA model of nucleotide substitution and rapid bootstrapping on the CIPRES Science Gateway (MILLER *et al.*, 2010). Support for phylogenetic relationships was assessed based on 1000 bootstrap replicates in RAXML and trees were viewed using FigTree (RAMBAUT, 2010). The trees are drawn to scale, with branch lengths measured in the number of substitutions per site.

Nomenclature. — All references to articles of ICN come from the Shenzhen Code (TURLAND *et al.*, 2018). Typifications and novelties were registered in the MycoBank Database (<http://www.mycobank.org>).

Definition of the genus *Tarzetia*

Since its reassessment at the beginning of the 1970s (ROGERS *et al.*, 1971; KORF, 1972), the genus *Tarzetia* was considered as a member

of the *Pyronemataceae*. The phylogenetic work by HANSEN *et al.* (2013) — based on a four-gene phylogeny — demonstrated that this family was paraphyletic and placed *Tarzetia* in a */tarzetia-geopyxis* lineage, a position confirmed by WANG *et al.* (2016) and KUMAR *et al.* (2017) with an enlarged dataset. BARAL (*in* JAKLITSCH *et al.*, 2016) resurrected the family *Otideaceae* erected by ECKBLAD (1968) and included *Tarzetia* as a member, while WIJAYAWARDENE *et al.* (2017) placed *Tarzetia* in *Pezizales incertae sedis* and EKANAYAKA *et al.* (2018), with a five-gene phylogeny, published the new family *Tarzettaceae*

Table 1 – List of sequenced collections for this study

Nom	Voucher Number	Host(s)	GenBank Accession #	
			ITS	LSU
<i>Hypotarzetia insignis</i>	LY:NV 2014.03.07	<i>Cedrus atlantica</i>	MN712290	MN712245
<i>Hypotarzetia insignis</i>	F.S. 2013002	<i>Pseudotsuga menziesii</i>	MN712291	MN712246
<i>Tarzetia alnicola</i>	LY:NV 2017.09.08	<i>Alnus</i> sp.	MN712299	MN712254
<i>Tarzetia alnicola</i>	LY:NV 2017.08.33	<i>Alnus</i> sp.	MN712300	MN712255
<i>Tarzetia alnicola</i>	LY:NV 2017.08.36	<i>Alnus incana</i>	MN712301	MN712256
<i>Tarzetia alnicola</i>	LY:NV 2008.07.06	<i>Alnus glutinosa</i>	MN712302	MN712257
<i>Tarzetia alpina</i>	LY:NV 2009.08.11	unknown	–	MN712259
<i>Tarzetia betulicola</i> ad int.	M.P. 2018-133	<i>Betula</i> , <i>Pinus uncinata</i>	–	MN712273
<i>Tarzetia bronca</i>	SAK-17-0423-5	<i>Populus</i> sp.	MN712304	MN712261
<i>Tarzetia catinus</i>	V.R. 20190509	<i>Fagus sylvatica</i> , <i>Quercus</i>	MN712315	MN712274
<i>Tarzetia catinus</i>	GM 20190505	<i>Fagus sylvatica</i> , <i>Quercus</i>	MN712316	MN712275
<i>Tarzetia catinus</i>	LY:NV 2007.04.18	<i>Fagus sylvatica</i> , <i>Corylus avellana</i> , <i>Picea abies</i>	–	MN712276
<i>Tarzetia catinus</i>	LY:NV 2002.06.05	<i>Fagus sylvatica</i> , conifers	–	MN712277
<i>Tarzetia catinus</i>	LY:NV 2010.05.09	<i>Fagus sylvatica</i>	MN712317	MN712278
<i>Tarzetia</i> cf. <i>catinus</i>	M.C. 18-10-13	<i>Castanea sativa</i> , <i>Quercus</i> sp.	MN712303	MN712258
<i>Tarzetia</i> cf. <i>catinus</i>	AH:53705	<i>Abies alba</i> , <i>Salix caprea</i> , <i>Corylus avellana</i>	MN712325	–
<i>Tarzetia cupularis</i>	LY:NV 2019.05.11	<i>Carpinus betulus</i> , <i>Quercus</i> , <i>Castanea sativa</i>	MN712319	MN712280
<i>Tarzetia cupularis</i>	MC 18-5-26	<i>Quercus pubescens</i> , <i>Castanea sativa</i>	MN712320	MN712281
<i>Tarzetia cupularis</i>	LY:NV 2013.11.24	hardwoods	MN712321	MN712282
<i>Tarzetia cupularis</i>	LY:NV 2006.10.31	<i>Quercus</i> , <i>Buxus sempervirens</i>	MN712322	MN712283
<i>Tarzetia cupularis</i>	LY:NV 2004.10.38	<i>Quercus</i> , <i>Pinus sylvestris</i>	MN712323	MN712284
<i>Tarzetia gregaria</i>	LY:NV 2017.08.16	unknown	MN712288	MN712243
<i>Tarzetia melitensis</i>	LY:CS871	<i>Quercus ilex</i> , <i>Laurus nobilis</i>	MN712324	MN712285
<i>Tarzetia ochracea</i>	LY:NV 2018.06.05	<i>Fagus sylvatica</i>	MN712308	MN712266
<i>Tarzetia ochracea</i>	M.C. 15-6-20	<i>Abies alba</i> , <i>Fagus sylvatica</i>	MN712309	MN712267
<i>Tarzetia ochracea</i>	LY:NV 2019.06.01	<i>Quercus</i> , <i>Populus tremula</i>	MN712310	MN712268
<i>Tarzetia ochracea</i>	LY:NV 2013.06.12	<i>Castanea sativa</i> , <i>Tilia</i>	MN712311	MN712269
<i>Tarzetia ochracea</i>	TaCu18061801	<i>Abies</i> sp., <i>Fagus sylvatica</i>	MN712312	MN712270
<i>Tarzetia ochracea</i>	LY:NV 2018.06.11	<i>Fagus sylvatica</i>	MN712313	MN712271
<i>Tarzetia ochracea</i>	LY:NV 2013.06.43	<i>Fagus sylvatica</i>	MN712314	MN712272
<i>Tarzetia pseudocatinus</i>	M.C. 18-5-21	<i>Crataegus</i>	MN712292	MN712247
<i>Tarzetia pseudocatinus</i>	C.A. 20121020.1	<i>Fagus sylvatica</i>	MN712293	MN712248
<i>Tarzetia pseudocatinus</i>	LY:NV 2019.05.12	<i>Carpinus betulus</i>	MN712294	MN712249
<i>Tarzetia pseudocatinus</i>	LY:NV 2014.08.19	<i>Corylus avellana</i>	MN712295	MN712250
<i>Tarzetia pseudocatinus</i>	LY:NV 2013.11.09	<i>Castanea sativa</i> , <i>Carpinus betulus</i>	MN712296	MN712251
<i>Tarzetia pseudocatinus</i>	LY:NV 2000.05.01	hardwoods	MN712297	MN712252
<i>Tarzetia pseudocatinus</i>	LY:NV 2006.09.01	<i>Carpinus betulus</i> , <i>Quercus</i>	MN712298	MN712253
<i>Tarzetia quercus-ilicis</i>	LY:NV 2014.03.20	<i>Quercus ilex</i>	MN712306	MN712264
<i>Tarzetia quercus-ilicis</i>	M.C. 14-3-15	<i>Quercus ilex</i>	MN712307	MN712265
<i>Tarzetia sepultarioides</i>	LY:NV 2017.08.03	<i>Picea abies</i> , <i>Alnus incana</i> , <i>Acer</i> sp.	MN712289	MN712244
<i>Tarzetia</i> sp.	M.C.-Sardinia	unknown	–	MN712260
<i>Tarzetia</i> sp.	LY:NV 2001.03.01	unknown (<i>Pinus</i> ?)	–	MN712262
<i>Tarzetia</i> sp.	LY:NV 2018.06.12	<i>Corylus avellana</i>	MN712305	MN712263
<i>Tarzetia</i> sp.	M.C. 08-5-23	<i>Quercus ilex</i>	MN712318	MN712279
<i>Tarzetia</i> sp.	LY:NV 2014.07.10	<i>Betula</i> , <i>Salix</i>	MN712326	MN712286

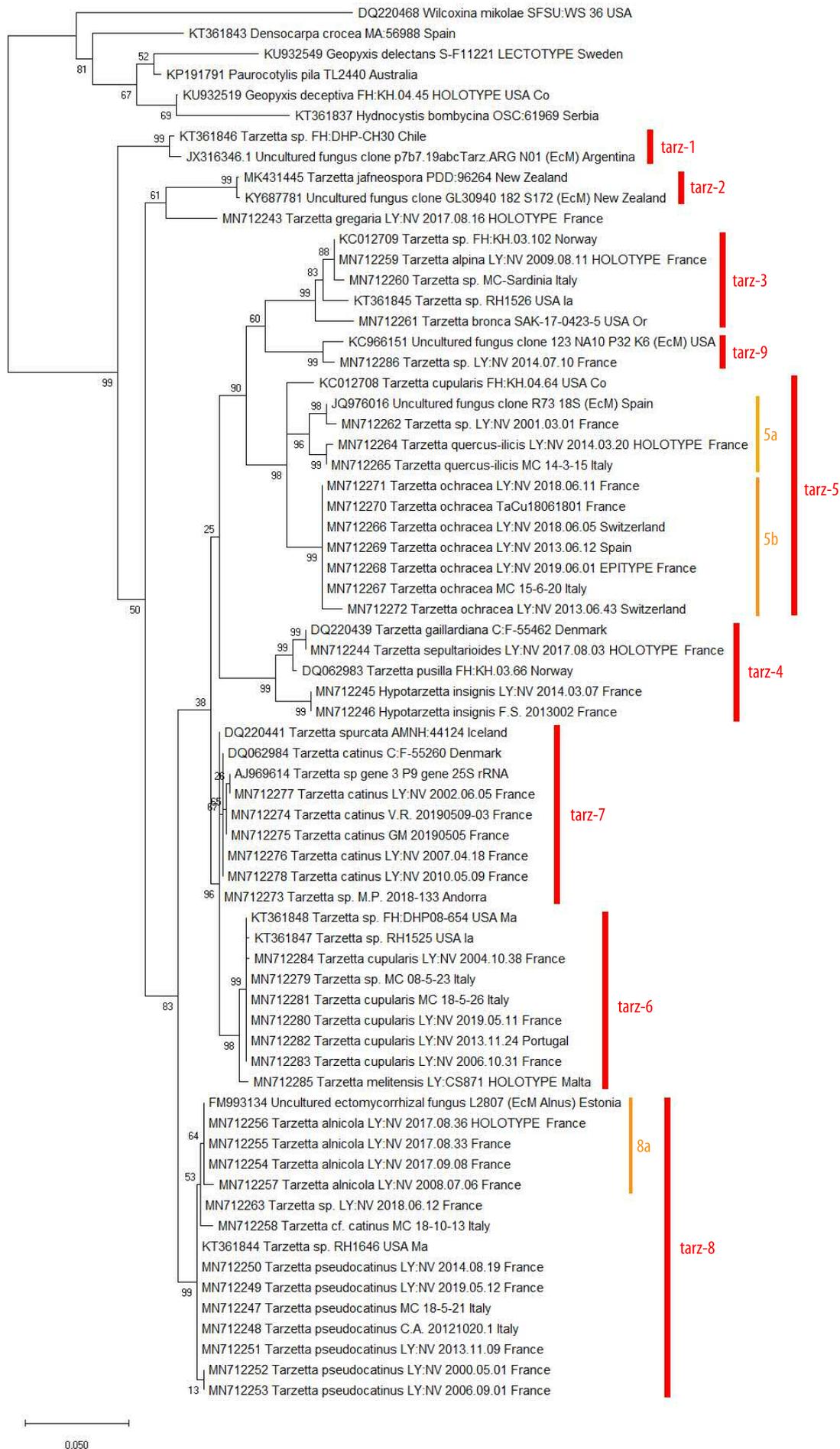


Fig. 1 – Molecular phylogenetic analysis by Maximum Likelihood method of LSU alignment of *Tarzetta*, rooted by other species of the /tarzetta-geopyxis lineage.

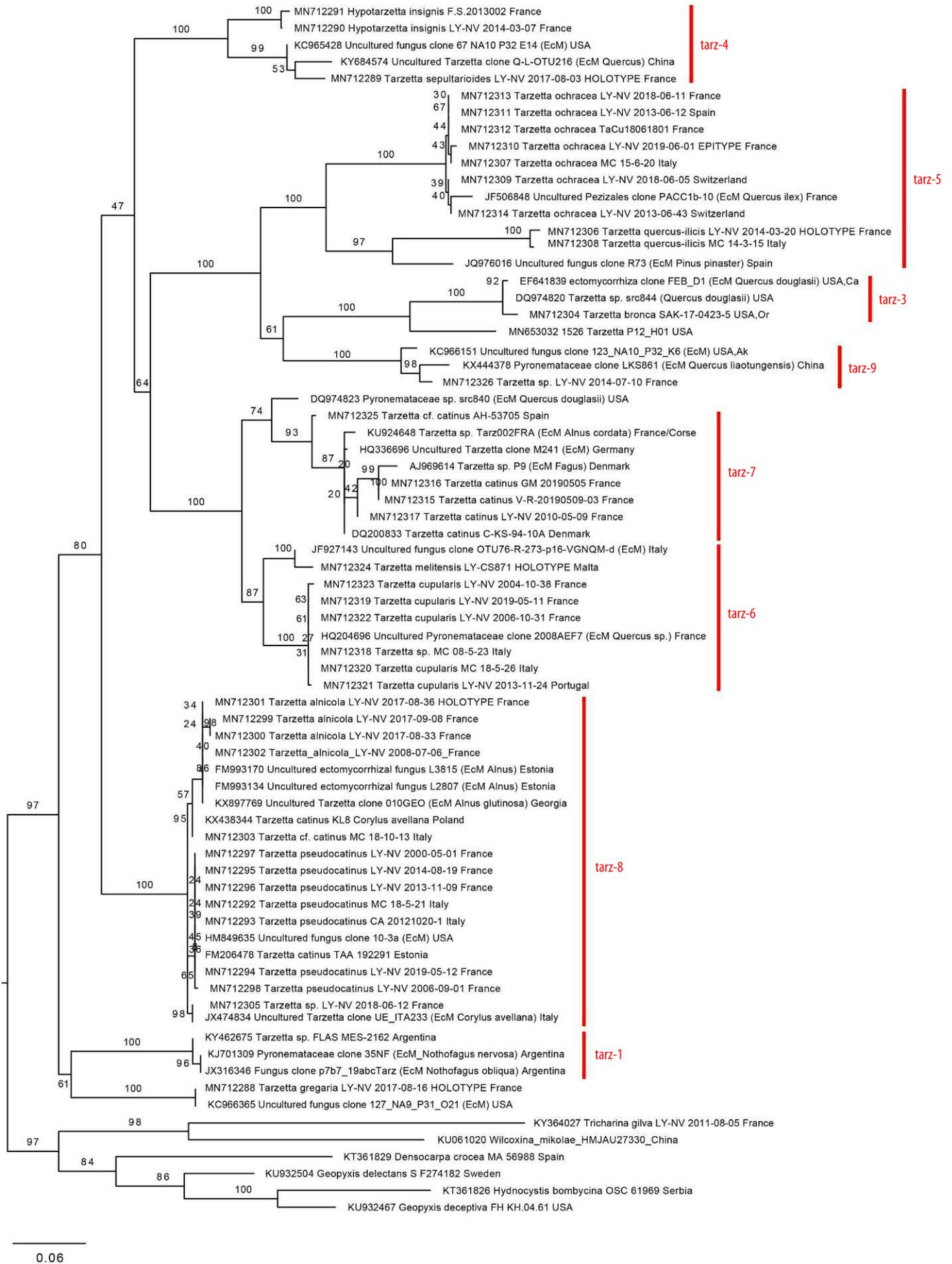


Fig. 2 – Molecular phylogenetic analysis by Maximum Likelihood method of ITS alignment of *Tarzetta*, rooted by other species of the /tarzetta-geopyxis lineage.

corresponding to the lineage /tarzetta-geopyxis as previously defined. This group also contains the apothecial genus *Geopyxis* (Pers.) Sacc. and the truffle-like genera *Hydnocystis* Tul. & C. Tul., *Paurocotylis* Berk. and *Densocarpa* Gilkey (KUMAR *et al.*, 2017).

Macromorphologically *Tarzetta* is characterised by apothecial ascomata, sessile or stipitate, deeply cupulate, grey to beige coloured, sometimes yellowish, ochraceous, rarely orangish, with an external surface pustulate at least when young; the hymenium is whitish or concolourous to the outer surface; the margin is often eroded or denticulate. Microscopically, the asci are operculate, often slightly curved at the top, inamyloid, without crozier. The paraphyses are septate, simple or \pm diverticulate at the top and contain nuclei staining red in acetocarmine solution. The ascospores are smooth or warty in one species, biguttulate, often accompanied by numerous internal droplets; this content is generally merged into one large oil drop on rehydrated material. The flesh is composed of two layers: a thick medullary excipulum of *textura intricata* and an ectal excipulum of *textura globulosa* to *angularis*; the outer part is often composed of aggregates of roundish cells which form macroscopic warts. Marginal cells are generally made of *textura prismatica*. Finally, the spore print is white.

Concerning the diagnostic characters for species, the size of apothecia is an important criterion. The presence of a stipe is also to be noted but its length can vary within the same collection and it can be buried in the substrate. Microscopically the size and shape of mature ascospores are important, but we believe that the spore content in the living state must be also considered because on rehydrated material the oil drops are merged. The top of the paraphyses was often used as an important diagnostic character — presence of diverticulate items or branchlets or bumps — to separate some species, but the situation seems more complex because we observed that this feature can vary within the same collection, depending on the age of the specimen or the area of hymenium observed. Thus, it does not appear to be a stable character. Finally, the ecology appears to be preponderant for many species and should be noted when collecting samples although it is not always easy to determine the possible tree (or plant) association.

Phylogenetic results

The analyses done on LSU and ITS sequences, respectively shown on Fig. 1 and 2, produced similar topologies. The monophyly of the genus *Tarzetta* is confirmed, including the monospecific genus *Hypotarzetta* Donadini. The main clades are strongly supported and give useful information on the hypothesized host-specificity of several taxa.

The clade /tarz-1 (Fig. 1) is represented by a collection made in Chile (FH:DHP-CH30, see KUMAR *et al.*, 2017) and an environmental sequence — with *Nothofagus obliqua* as host (JX31634) — from Argentina. On Fig. 2, a similar clade is represented and the collection FLAS:MES-2162 may represent another species, also associated with *Nothofagus* (*N. antarctica* or *N. dombeyi* based on the collector data). These collections should be compared with *Tarzetta microspora* (Raitheh.) Raitheh. (= *Pustularia microspora* Raitheh., 1983), a species described from Argentina. *Tarzetta jafneospora* probably also associated with *Nothofagus* spp., appears in its own clade.

The clade /tarz-2 (Fig. 1 only) corresponds to *T. jafneospora*, an endemic species from New Zealand. *Tarzetta jafneospora* could be associated with *Nothofagus* spp., a dominant canopy tree. As a “sister” branch, there is a new species, *T. gregaria* (see under this name in the chapter on Taxonomy).

The clade /tarz-3 comprises the new species *T. alpina* (see under this name), and two North-American collections, one tentatively determined as *T. bronca* (Peck) Korf & J.K. Rogers and another one probably new to science, growing in an oak dominated woodland, with *Q. alba* and *Q. macrocarpa* (HEALY, pers. comm.).

The clade /tarz-4 comprises two robust subclades: 1) *Hypotarzetta insignis* (Berthet & Rioussset) Donadini, a semi-hypogeous species;

this demonstrates that *Hypotarzetta* is nested within the *Tarzetta* lineage and the correct name is *Tarzetta* rather than *Hypotarzetta* as proposed by DONADINI (1985). Originally the species was published in the genus *Pustularia*, the genus name used by mycologists before the resurrection of *Tarzetta*. 2) Two species, i.e. *T. pusilla* and *T. sepultarioides* sp. nov., discussed in the next chapters.

The clade /tarz-5 is divided into two subclades: ‘5a’ corresponds to the sequences identified as *Tarzetta ochracea* (see next chapter); ‘5b’ contains a group of Mediterranean taxa in which we describe *T. quercus-ilicis*, a new species associated with *Quercus ilex*. See the comments under this name. Another collection, from Colorado, USA, determined as *T. cupularis*, occupies an isolated position within /tarz-5 and requires further investigation.

The clade /tarz-6 has been chosen to represent *Tarzetta cupularis* (see discussion in the next chapter). A collection made in Malta occupies an isolated position and is described herein as *T. melitensis* sp. nov.

The clade /tarz-7 has been chosen to represent *Tarzetta catinus* (see discussion in the next chapter). It forms a complex of species with large apothecia, mainly associated with *Fagus* spp. More genes are required to circumscribe the different species. We tentatively delimited two collections made under *Betula*, the first one determined as *T. spurcata* (AMNH:44124) and the second one under the provisional name *T. betulicola* (see under this name).

Based on the hypothesis that collections represented in the /tarz-7 include the original concept of *Tarzetta catinus*, the clade /tarz-8 may represent a complex of species often determined as *T. catinus*. Within this complex, the subclade 8a is named *T. alnicola* sp. nov., a species associated with alders. The host tree for other collections, provisionally named *T. pseudocatinus*, is not clearly identified (*Carpinus betulus* is noted for 3 collections, *Corylus avellana* = 1, *Fagus sylvatica* = 2, *Pinus* sp. = 1, *Castanea sativa* = 2, *Quercus* sp. = 1, *Crataegus* sp. = 1 and “hardwoods” = 1). The sequenced collections do not differ significantly from *T. catinus* and we do not find any difference in spore size or shape. Further investigations are required to explore this species complex and circumscribe correctly the species where there is unambiguous evidence to. One of these taxa appears to be strictly associated with *Corylus* spp. (represented by the collection LY:NV 2018.06.12 and an ITS sequence from an environmental sample, GenBank JX474834).

Finally, one of our collections (LY:NV 2014.07.10) is included in the clade /tarz-9 with two environmental ITS sequences (Fig. 2), one isolated from roots of *Quercus liaotungensis*, in China (GenBank KX444378), and one isolated from soil in the Arctic part of Alaska (GenBank KC966151; TIMLING *et al.*, 2014). Our collection comes from the French Alps, at 1190 m elev., and was growing along a small brook, close to *Betula* and *Salix* trees. Nothing can be here concluded, and more collections are needed.

Review of existing names and typifications

Tarzetta catinus (Holmsk.) Korf & J.K. Rogers, in Korf, *Phytologia*, 21 (4): 206 (1971). Plates 1–2 and fig. 5A.

Basionym: *Peziza catinus* Holmsk., *Beata ruris otia fungis danicis*, 2: 22 (1799); sanctioned by Fries, *Syst. mycol.*, 2: 61 (1822).

Original diagnosis: “stipitata, globoso-campanulata; limbo patente, repando; superficie externa pallide ochracea, punctis fuscis notata, interna glabra, ex fusco ochracea.”

Lectotype here designated: Plate 8, HOLMSKJOLD (1799); MBT 389442.

Homotypic synonyms: *Peziza sphaelata* subsp. *catinus* (Holmsk.) Pers., *Mycol. Europ.*, 1: 231 (1822); *Pustularia catinus* (Holmsk.) Fuckel, *Jahrb. Nass. Verein. Naturk.*, 23–24: 328 (1870); *Aleuria catinus* (Holmsk.) Gillet, *Champ. Fr., Discom.*: 39 (1879); *Geopyxis catinus* (Holmsk.) Sacc., *Syll. fung.*, 8: 71 (1889); *Pustulina catinus* (Holmsk.) Eckblad, *Nytt Mag. Bot.*, 15: 84 (1968).



Plate 1 – *Tarzetta catinus*. A: Lectotype of *Peziza catinus* from HOLMSKJOLD (1799, pl. 8). B: Coll. G.M. 2019 05 05; photo G. Moyne. C: Coll. V.R. 20190509; photo V. Ricard. D: Coll. LY:NV 2007.04.18 (young specimens); photo N. Van Vooren. E: Coll. AH:53705; photo L. Sánchez.

Taxonomical synonyms: *Peziza tarzetta* Cooke, *Mycographia*, 1: 166 (1877); *Discina pallida* Velen., *Česke Houby*, 4-5: 860 (1922), *vide* SVRČEK (1979).

Peziza catinus is lectotypified by the beautiful plate depicted by HOLMSKJOLD (1799, pl. 8). It is part of the material cited by FRIES (1822) in his sanctioning publication *Systema mycologicum*. This plate shows several specimens at different stages of development. This species has a rather long stipe compared to the other species and large apothecia. FRIES (*op. cit.*) indicated: “*Stipes praecedentis longior, constanter praesens, firmus, 2 lin. fere crassus, subglaber. Cupula [...]*

1-2 *unc. lata*.” This suggests that the stipe usually measures about 4 mm diam. and the apothecium is between 25 to 50 mm¹ diam. The plate 2 illustrates an authentic Fries’ collection housed in UPS which perfectly matches his description. This concept was followed by BOUDIER (1905-1910, under *Pustularia catinus*), NANNFELDT (1938, under *P. catinus*), DENNIS (1978), DISSING *et al.* (2000), YAO & SPOONER (2002) and VAN VOOREN (2014). Our phylogenetic analyses suggest this concept is not homogeneous regarding the morphology. The designation of an epitype based on a recent and well-documented collection coming from Denmark, near Aarhus (where the original “collection” was found) is urgently needed.

¹ lin. = *linea*, which means “line”, an ancient unit of length. The value was not the same in all countries, but in Sweden before 1879 one line corresponded to about 2 mm after J. Melot (*comm. pers.*). The same applied to “unc. = *uncia*” which means “once” (inch), about 25 mm.

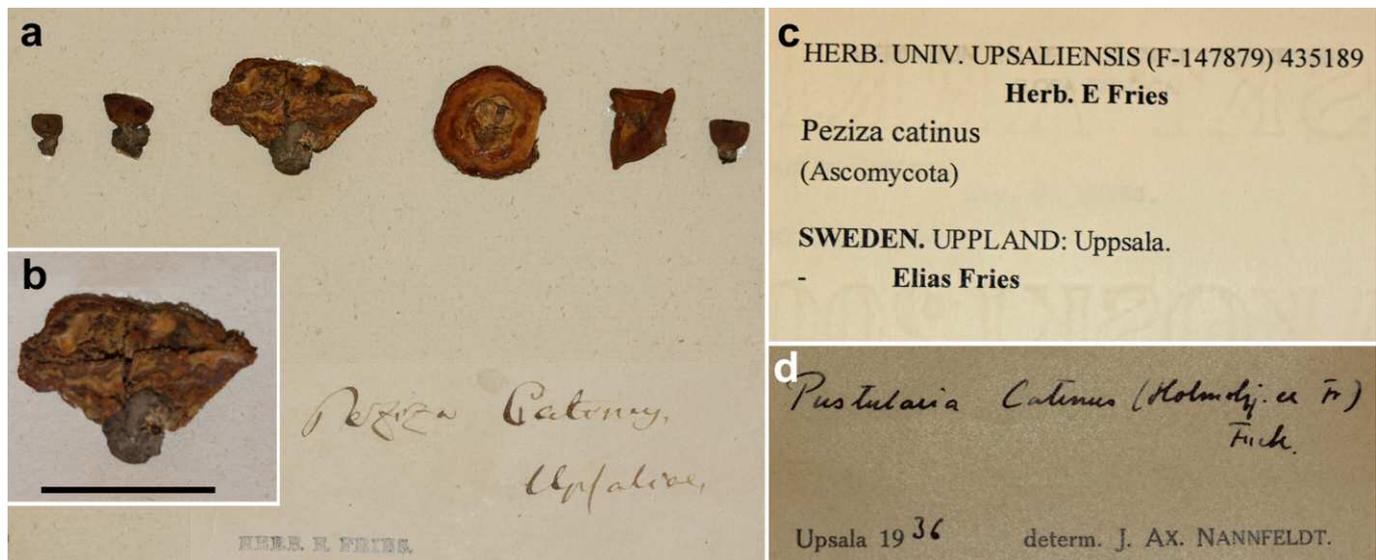


Plate 2 – *Tarzetta catinus*. Collection of *Peziza catinus* from Fries herbarium. a: dried specimens. b: close-up view of a specimen (scale bar = 20 mm). c: Label. d: Nannfeldt's label. All photos Åsa Krusys (UPS).

Waiting for the opportunity to study such collection, we select the collection C:F-55260 ex KS-94-10 [GenBank ITS DQ200833; LSU DQ062984; RPB2 JX943768] from Denmark (TEDERSOO *et al.*, 2006; HANSEN *et al.*, 2013) to represent *T. catinus*. It likely forms ectomycorrhizae with beech (*Fagus sylvatica*). This hypothesis seems to be correct based on the other collections or EcM data of the /tarz-7 clade shown in Fig. 1 & 2.

Tarzetta pseudocatinus (provisional name; Plate 3 and fig. 5D) is morphologically very close to, but molecularly distant from *T. catinus*. The morphological criteria to distinguish them could be hard to establish. The ecological data may prove helpful once the correct hosts have been identified. It is also possible that this taxon has developed interactions with several plants.

Studied material: *Tarzetta catinus*. – FRANCE: Isère, Lans-en-Vercors, Col de la Croix Chabaud, 45.10576° N 5.54601° E, 1215 m a.s.l., on soil, under *Fagus sylvatica* and conifers, 15 Jun. 2002, leg. M. Poizat, herb. NV 2002.06.05 (LY). Aude, Belcaire, Clos de la Plaine, 42.841328° N 1.954536° E, 980 m a.s.l., on soil, under *Picea abies*, *Fagus sylvatica* and *Corylus avellana*, 30 Apr. 2007, leg. N. Van Vooren, herb. NV 2007.04.18 (LY). Loire, Saint-Georges-en-Couzan, pont du Diable, 45.6954455° N 3.9356588° E, 560 m a.s.l., on soil, under *Fagus sylvatica*, 5 May 2010, leg. N. Van Vooren, herb. NV 2010.05.09 (LY). Doubs, Amancey, le Grand Bois, ~47.03925° N 6.08879° E, 595 m a.s.l., on soil, under *Fagus sylvatica* and *Quercus* sp., 21 May 2019, leg. G. Moyne, pers. herb. G.M. 2019 05 05. Haute-Marne, Ormancey, forêt domaniale, 47.919962° N 5.160481° E, 430 m a.s.l., on soil under *Fagus sylvatica*, *Quercus* sp. and *Carpinus* sp., 9 May 2019, leg. V. Ricard, pers. herb. V.R. 20190509-03. SPAIN: Catalonia, Riu de Cerdanya, Prat de les Cabanes, 42.323675° N 1.8184611° E, 1550 m a.s.l., along a stream, on soil, under *Abies* sp., *Salix caprea* and *Corylus avellana*, 28 Jun. 2014, leg. L. Sánchez, herb. 53705 (AH). ***Tarzetta pseudocatinus* agg.** – FRANCE: Rhône, Les Haies, Col de Croix-Régis, 45.52189° N 4.74278° E, 500 m a.s.l., on soil, under hardwoods, 1 May 2000, leg. H. Orcel, herb. NV 2000.05.01 (LY). Rhône, La Tour de Salvagny, Cerqueminal, 45.8077383° N 4.7014671° E, 275 m a.s.l., on soil, under *Carpinus betulus* and *Quercus* sp., 4 Sep. 2006, leg. N. Van Vooren, herb. NV 2006.09.01 (LY). Rhône, Saint-Priest, bois de Feuilly, 45.71302° N 4.92475° E, 210 m a.s.l., on soil, under *Castanea sativa* and *Carpinus betulus*, 7 Nov. 2013, leg. J. Cavet, herb. NV 2013.11.09 (LY). Hautes-Pyrénées, Aulon, Granges de Lurgues, 42.85388° N 0.27167° E, 1445 m a.s.l., on soil, under hardwoods, mainly *Corylus avellana*, 26 Aug. 2014, leg. N. Van Vooren, herb. NV 2014.08.19 (LY).

Seine-et-Marne, Vaux-le-Pénil, parc Faucigny-Lucinge, 48.5367° N 2.66905° E, 80 m a.s.l., on soil in an urban park, under *Carpinus betulus*, 29 May 2019, leg. R. Chalange, herb. NV 2019.05.12 (LY). ITALY: Caserta, Piano della Corte, ~41.463333° N 14.394166° E, 1400 m a.s.l., on soil, under *Fagus sylvatica*, 20 Oct. 2012, leg. A. Conte, pers. herb. C. Agnello C.A. 20121020.1. Lombardy, Milan, Paderno Dugnano, Parco Toti, 45.5715° N 9.175333° E, 170 m a.s.l., on soil, under *Crataegus* sp., 21 May 2018, leg. E. Sacchi, pers. herb. M.C. 18-5-21 (duplicata in LY). Liguria, Ruta di Camogli, 44.348944° N 9.17775° E, 245 m a.s.l., on soil, under *Castanea sativa* and *Quercus* sp., 13 Oct. 2018, leg. M. Carbone, pers. herb. M.C. 18-10-13 (duplicata in LY).

***Tarzetta cupularis* (L.) Lambotte**, *Mém. Soc. roy. sci. Liège, série 2*, 14: 325 (1888). Plate 4 and fig. 5B.

Basionym: *Peziza cupularis* L., *Syst. Plant.*, 2: 1181 (1753); sanctioned by Fries, *Syst. mycol.*, 2: 62 (1822).

Type: "neotype" designated by PANT & TEWARI (1971), collection Fuckel's Fungi Rhenani no. 1878, housed in Royal Botanic Gardens, Kew (K). See comments.

Lectotype here designated: *Bot. paris.*, pl. 11, fig. 1-3, VAILLANT (1727); MBT 389443.

Original diagnosis: "*Peziza globoso-campanulata: margine crenato.*"

Peziza calice campanulato globoso: ore crenato. Guett. *Stamp.* 16. Dalib. *paris.* 388.

Fungoides glandis cupulam referens, margine dentato. Vaill. *paris.* 57. t. 11. f. 1. 2. 3.

Habitat in Gallia."

Homotypic synonyms: *Pustularia cupularis* (L.: Fr.) Fuckel, *Jahrb. Nass. Verein. Naturk.*, 23-24: 328 (1870); *Aleuria cupularis* (L.) Gillet, *Champ. Fr., Discom.*: 39 (1879); *Geopyxis cupularis* (L.) Sacc., *Syll. fung.*, 8, p. 72 (1889); *Pustulina cupularis* (L.) Eckblad, *Nytt Mag. Bot.*, 15: 85 (1968).

Taxonomical synonyms: *Peziza crenata* Bull., *Herb. Champ. Fr.*, pl. 396, fig. III (1786); *Geopyxis grossegranulosa* Velen., *Novit. Mycol. Novissim.*: 152 (1947), *fide* SVRČEK (1979).

PANT & TEWARI (1971) designated a neotype based on Fuckel's Fungi Rhenani no. 1878 collection (from Hessen in Germany), housed in Royal Botanic Gardens, Kew (K). Unfortunately, this designation is not acceptable because the authors did not respect the rules for typification, especially Art. 9.7 and 9.10 of ICN. As the

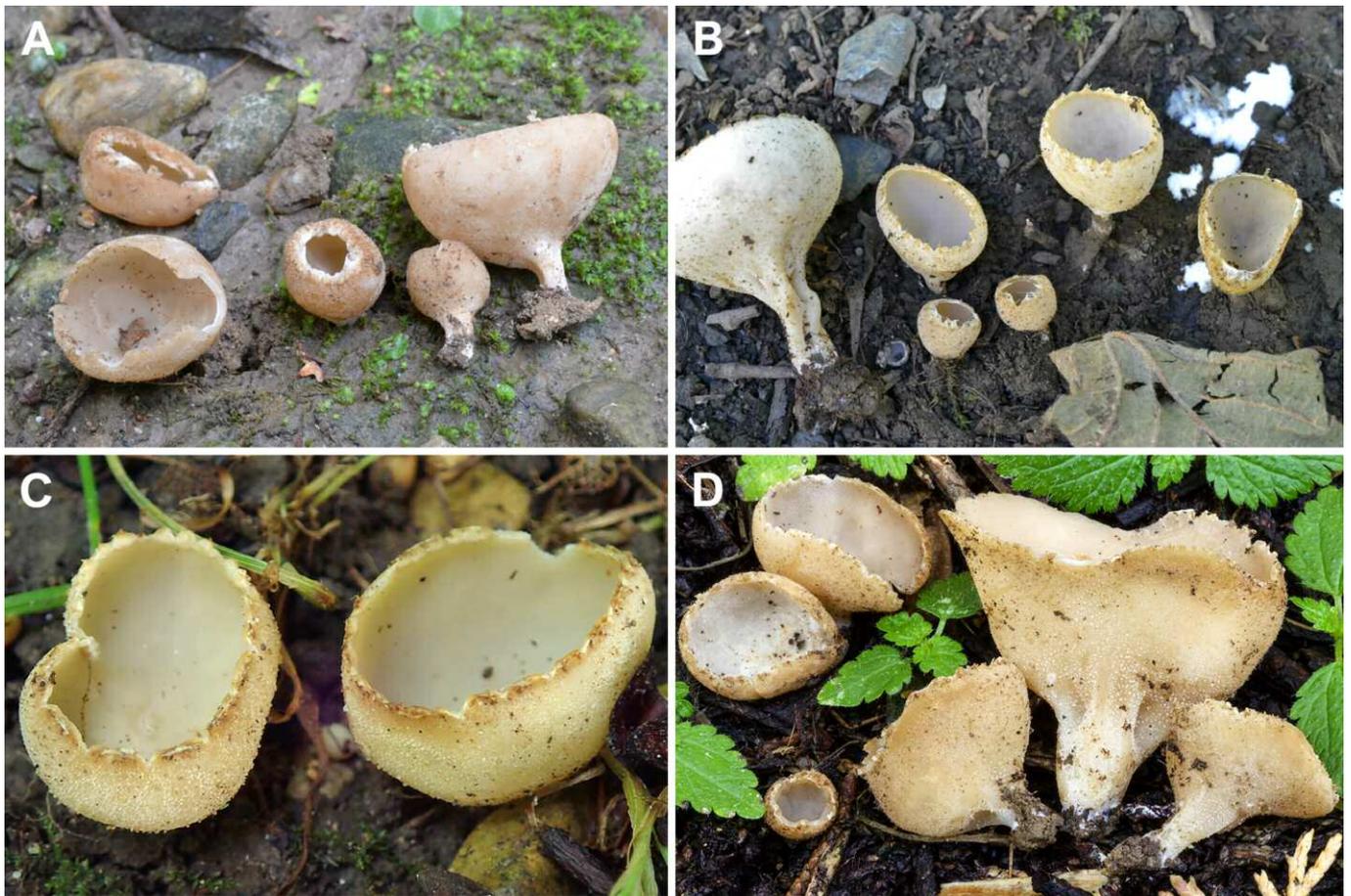


Plate 3 – *Tarzetta pseudocatinus* agg. A: Coll. LY:NV 2013.11.09; photo N. Van Vooren. B: Coll. LY:NV 2014.08.19; photo N. Van Vooren. C: Coll. LY:NV 2019.05.12; photo M.-P. Vigneron. D: *Tarzetta* cf. *catinus* - coll. M.C. 18-10-13; photo M. Carbone.

species was originally described by LINNÉ (1753) from France, we designated a lectotype based on Vaillant's drawing (1727), listed by LINNÉ (*op. cit.*) and by FRIES (1822) in agreement with Art. 9.10 for sanctioned names. This drawing is here reproduced on the plate 3. Of course, this lectotype is not sufficient to clearly circumscribe this name because no microscopical or molecular data are available from this original material. The species is often considered as a sessile or short-stipitate species (as illustrated by VAILLANT, 1727). FRIES (1822) indicates the ascocarps are "3–8 lin. lata", which is approximately 6.8–18 mm, and is thus considered as a rather small species for the genus. A French collection made near Versailles² (or in the south of Paris), growing under hardwoods and possessing such characters is actively searched to provide a modern definition of this taxon and designate an epitype. In the meantime, we have selected a clade containing collections which fit well this definition. In this clade, the collections are probably associated with oaks (*Quercus* spp.) and show a wide distribution (presence in Europe and USA).

Tarzetta scotica (Rea) Y. J. Yao & Spooner is considered by YAO & SPOONER (2002) as a species close to *T. cupularis* mainly differing by smaller ascospores. After the original description (REA, 1928), this species is sessile. No information is given about the ecology of this taxon first collected near Perth, on the eastern coast of Scotland. We did not yet have the opportunity to study *T. scotica* and evaluate its characters to make a conclusion.

Studied material: FRANCE: Rhône, La Tour-de-Salvagny, les Engrollets, 45.8025517° N 4.7011771° E, 310 m a.s.l., on burnt ground, under *Quercus* sp. and *Pinus sylvestris*, 21 Oct. 2004, leg. N. Van

Vooren, herb. NV 2004.10.38 (LY). Rhône, Saint-Romain-au-Mont-d'Or, Mont Thoux, 45.83935° N 4.807991° E, 420 m a.s.l., on soil, under *Quercus* sp. and *Buxus sempervirens*, 24 Oct. 2006, leg. N. Van Vooren, herb. NV 2006.10.31 (LY). Rhône, La Tour-de-Salvagny, Cerqueminal, 45.8077383° N 4.7014671° E, 275 m a.s.l., on soil, under *Carpinus betulus*, *Quercus* sp. and *Castanea sativa*, 31 May 2019, leg. N. Van Vooren, herb. NV 2019.05.11 (LY). PORTUGAL: Viseu, Parque de Fontelo, 40.659155° N 7.898876° W, 475 m a.s.l., on soil under hardwoods, 22 Nov. 2013, leg. N. Van Vooren, herb. NV 2013.11.24 (LY). ITALY: Piedmont, Variano, 44.7202778° N 8.938611° E, 330 m a.s.l., on soil, under *Quercus pubescens* and *Castanea sativa*, 26 May 2018, leg. M. Carbone, duplicata herb. M.C. 18-5-26. Liguria, Mezzema, Deiva Marina, 44.252583° N 9.548555° E, 500 m a.s.l., on soil, under *Quercus ilex*, 23 May 2008, leg. M. Carbone, pers. herb. M.C. 08-5-23.

Tarzetta gaillardiana (Boud.) Korf & J.K. Rogers, in Korf, *Phytologia*, 21 (4): 206 (1971). Fig. 3.

Basionym: *Pustularia gaillardiana* Boud., *Bull. Soc. mycol. France*, 18: 141 (1902).

Type: holotype in PC, ex herb. Boudier; isotype, no. 28 in herb. Gaillard (Musée botanique d'Angers, Arboretum).

Original diagnosis: "*Pustularia gaillardiana* Boud. (Pl. 8, fig. 1).

Minuta, 4–8 mm lata, cupularis, cinereo-fulva, brevissime stipitata aut sessilis, extus parce tomentella margine subcrenulato. Receptaculum cupulare, minute tomentello-granulosum, margine subcrenulato, brevissime stipitatum, griseo-fuscum, hymenio concolore laeve. Thecae cylindricae, ad basim sub-attenuatae, hyalinae, operculatae, octosporae.

² In the original VAILLANT'S text, this author said: "Il croit dans les bosquets de Versailles vers la fin d'Aoust & le commencement de Septembre." which means "It grows in the groves of Versailles from the end of August to the beginning of September."

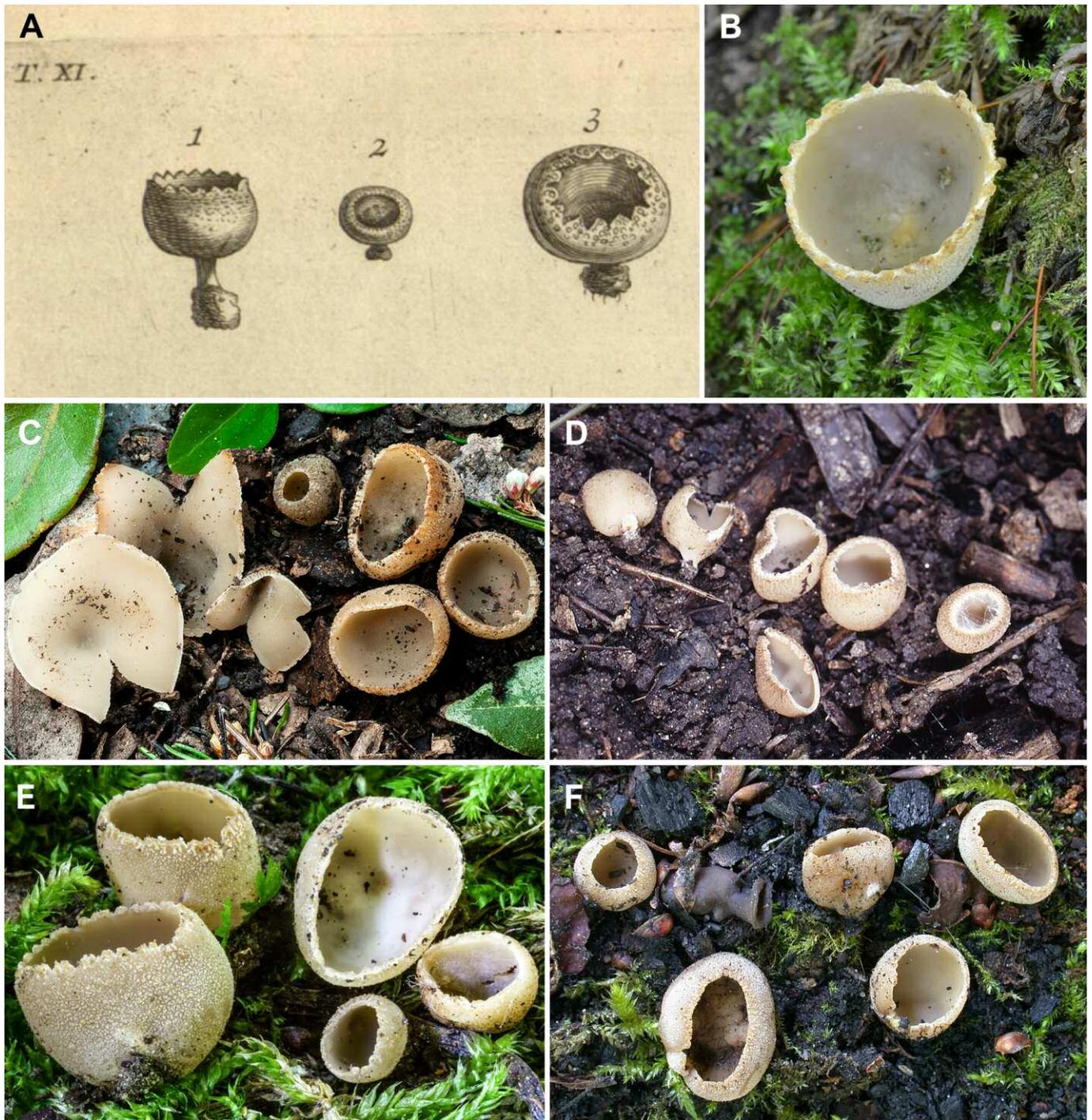


Plate 4 – *Tarzetta cupularis*. A: Lectotype of *Peziza cupularis* from VAILLANT (1727, pl. 11, fig. 1-3). B: Coll. LY:NV 2019.05.11; photo N. Van Vooren. C: Coll. M.C. 08-5-23; photo M. Carbone. D: Coll. LY:NV 2004.10.38 (on burnt ground); photo N. Van Vooren. E: Coll. M.C. 18-5-26; photo M. Carbone. F: Coll. LY:NV 2012.05.43 (on burnt ground).

rae, 300–320 μ longae, 15–20 *crassae*. *Paraphyses* *tenues*, *hyalinae*, *septatae*, *intus vix granulosae ad apicem paululum incrassatae*, 3–4 μ *spissae*. *Sporae* *majores*, *ellipticae*, *laeves*, *intus guttulis duobus oleosis*, *majoribus et granulis primo sat numerosis dein maturitate rarioribus aut deficientibus repletae*. *Long.* 25–27 μ . *Latitud.* 13–14.

Angers, *ad terram arenosam in nemoribus legit* Novembre 1901 *clar.* D. Gaillard."

Homotypic synonyms: *Geopyxis gaillardiana* (Boud.) Sacc. & D. Sacc., *Syll. fung.*, 18: 15 (1906); *Pustulina gaillardiana* (Boud.) D.C. Pant & V.P. Tewari, *Mycologia*, 62 (6): 1191 (1971).

Taxonomical synonyms: *Geopyxis albocinerea* Velen., *Novit. Mycol. Novissim.*: 152 (1947), *fide* SVRČEK (1979); *Geopyxis cavinae* Velen., *Česke Houby*, 4-5: 859 (1922), *fide* SVRČEK (1979); *Geopyxis*

cupularis Velen., *Česke Houby*, 4-5: 859 (1922), *fide* SVRČEK (1979); *Geopyxis patellaris* Velen., *Monog. Discom. Bohem.*: 336 (1934), *fide* SVRČEK (1979).

The isotype has been reviewed and possesses ascospores shorter than those described by BOUDIER (1902), † (20) 21–24 \times (11) 11.5–14 μ *m* vs. 25–27 \times 13–14 μ *m*, with a Q value between 1.6 and 2.1 (mean 1.8). PANT & TEWARI (1970) revised the type collection housed in PC and they found ascospores measuring 13–19 \times 6.5–11 μ *m* (*sic!*), but they do not discuss this important difference with the prologue. The desiccation process cannot explain such a difference in spore length. We cannot exclude a difference of maturity between the specimens examined by Boudier or the fact he measured some

overmature ascospores. Unfortunately, our attempts to obtain a DNA sequence from the isotype failed.

SVRČEK (1979) listed several Velenovský's names as synonyms of *T. gaillardiana* but it seems difficult to evaluate their relevance without a reexamination of these collections or a DNA analysis.

Finally, new collections³ from the type locality, near Angers (France), are required to fix a modern interpretation of this species.

Studied material: FRANCE: Maine-et-Loire, Saint-Sylvain-d'Anjou, "La Baronnerie", 15 Nov. 1901, leg. D. Gaillard, coll. no. 28 in herb. Gaillard, isotype (Musée botanique d'Angers, Arboretum).

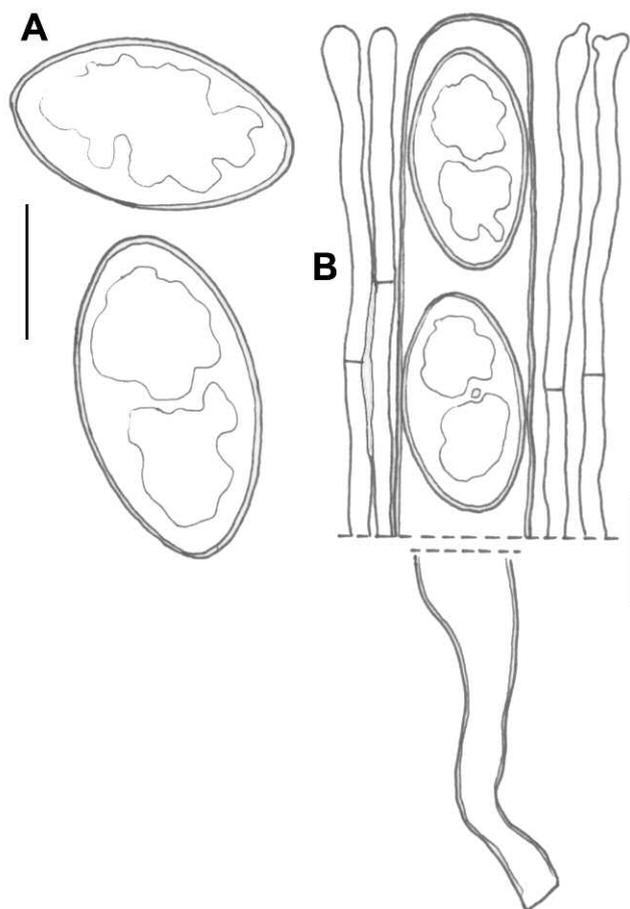


Fig. 3 – *Tarzetta gaillardiana* (isotype). A: Ascospores. B: Paraphyses and ascus. Scale bars = 10 μ m. Drawing N. Van Vooren

Tarzetta insignis (Berthet & Riousset) Korf & J.K. Rogers, in Korf, *Phytologia*, 21 (4): 206 (1971). Plate 5.

Basionym: *Pustularia insignis* Berthet & Riousset, *Bull. Soc. mycol. France*, 79: 397 (1963).

Original diagnosis: "Apotheciis primum cupulatis pedunculatisque, deinde subsessilibus, late explanatis, exterius convolutis, saepe pliacatis, 6–12 cm maturescentibus. Margine semper reflexa, primum integra, deinde incisa. Hymenio pallido, albo-flavescente. Facie externa concolore, sed paulum atriore, dense furfuracea. Carne tenue, firma, flexile, alba. Ascis longe cylindricis, octosporis, 390–410 \times 18–20 μ . Sporis ellipticis, laevibus, maturitate duabus magnis guttulis fere totis impletis, 25–27 \times 14–15 μ . Paraphysibus filiformibus, linearibus, septatis, saepe, ramosis, anastomosantibus, ascos longitudine aequan-

tibus, 2,5–3 μ latis. Sparsis in terra argilacea humosa, in cedreto. Typo in Ly sub n° 346."

Type: holotype in LY, ex herb. Berthet PB 346.

Homotypic synonyms: *Pustulina insignis* (Berthet & Riousset) Korf & Berthet, *Natur. Canad.*, 96: 248 (1969); *Hypotarzetta insignis* (Berthet & Riousset) Donadini, *Doc. mycol.*, 15 (60): 49 (1985).

We sequenced one of our collections coming from the type locality in the French Massif of Luberon (BERTHET & RIOUSSET, 1963). Our analysis confirms the placement in *Tarzetta* and the combination in the monotypic genus *Hypotarzetta* by DONADINI (1985) is therefore superfluous. This species was initially associated with cedar trees, i.e. *Cedrus atlantica*, but it was recently discovered in Spain under pine trees, i.e. *Pinus sylvestris* (MATEO FERNÁNDEZ, 2011) and in north-eastern France under Douglas fir, *Pseudotsuga menziesii* (SARRAILLON & DOLL, 2013). The latter collection was sequenced and its ITS is 100% identical to the ITS obtained from the "topotype" collection. This result suggests *T. insignis* is not exclusively associated with cedar trees, but also with other *Pinaceae* species.

T. insignis possesses the largest ascospores of the European *Tarzetta*, sometimes reaching 29 μ m in length.

Ascomata initially hypogeous, emerging when mature. **Apothecia** 45–85 mm diam., sessile or very shortly stipitate, irregularly cupuliform or strongly deformed, with a whitish, creamy or yellowish grey, velvety hymenium; external surface concolorous, strongly pustulate. **Margin** curved, eroded. **Flesh** thick, brittle.

Medullary excipulum 800–1100 μ m thick, of *textura intricata*, with hyaline hyphae. **Ectal excipulum** rather thick, ~250 μ m, made of clavate cells, 17–35 μ m wide, mixed with some globose, subglobose or angular elements; presence of emerging hyaline hyphae, septate, reaching 130 μ m in length. **Asci** (330) 370–400 \times 18–20 μ m, cylindrical, long narrowed to the base, without crozier, operculate, inamyloid, 8-spored. **Paraphyses** filiform, 3–4 μ m diam., not enlarged at the top, straight or flexuous, simple or sometimes forked, often with lateral bumps or appendages, hyaline. **Ascospores** (22) 23–27 (29) \times (12.5) 13–17 μ m [$X = 25.3 \times 13.9 \mu$ m, $n=51$], $Q=1.6$ –2.1 (2.3), $Q_m=1.8$, ellipsoid, sometimes slightly tapered at the ends, hyaline, smooth, rather thin-walled, containing two large oil drops, accompanied with numerous small guttules.

Studied material: FRANCE: Vaucluse, Bonnieux, massif des Cèdres, 43.801115° N 5.2712607° E, 705 m a.s.l., on soil, under *Cedrus atlantica* and *Buxus sempervirens*, leg. N. Van Vooren, 14 Apr. 2007, herb. N.V. 2007.04.08 (LY); *idem*, under *C. atlantica* and *Pinus* sp., leg. D. Borgarino, 3 May 2008, herb. N.V. 2008.05.02 (LY); *idem*, leg. N. Van Vooren, 2 May 2009, herb. N.V. 2009.05.02 (LY); *idem*, leg. N. Van Vooren, 16 May 2010, herb. N.V. 2010.05.23 (LY); *idem*, leg. N. Van Vooren, 16 May 2010, herb. N.V. 2010.05.23 (LY); Lacoste, massif des Cèdres, 43.80149° N 5.26823° E, 710 m a.s.l., on soil, under *C. atlantica*, leg. N. Van Vooren, 21 Mar. 2014, herb. N.V. 2014.03.07 (LY); Haut-Rhin, Osenbach, Bickenberg, 560 m a.s.l. on soil, under *Pseudotsuga menziesii*, leg. D. Doll, 9 Jun. 2013, duplicata herb. F.S. 2013002. GREECE: West Macedonia, Grevena, Ziakas-Perivolaki, 40.035378° N 21.245003° E, 950 m a.s.l., on soil, under *Abies borisii-regis*, leg. G. Konstantinidis, 31 Mar. 2007, duplicata herb. G.K. 2094.

Tarzetta ochracea (Boud.) Van Vooren, *comb. nov.* – MB 833284. Plate 6 and fig. 5E.

Basionym: *Pustularia ochracea* Boud. ex Boud., *Icon. mycol., sér. I, livr. 2*, pl. 337 (1904), a new name based on *Peziza ochracea* Boud., in Cooke, *Mycographia*, 5: 225 (1879), *illeg.*

Type: no type specimen is housed in Cooke's herbarium at K (DAVIES, pers. comm.).

³ A collection cited in MORNAND & PÉAN (1995) was revised and corresponds to a *Sepultaria* species (confirmed by a DNA sequence).



Plate 5 – *Tarzetta insignis*. A: Coll. LY:NV 2014.03.07. B: Ascospores from coll. LY:NV 2010.05.23. C: Coll. LY:NV 2007.04.08. D: Coll. LY:NV 2009.05.02 (detail of the outer surface). All photos N. Van Vooren.

Lectotype here designated: Fig. 377, *Peziza ochracea*, COOKE (1879); MBT 389444.

Epitype here designated: France, Essonne, forêt de Rougeau, LY:NV 2019.06.01; MBT 389445.

Original diagnosis: “*Caespitosa, integra, subflexuosa, laete ochracea, extus furfuracea. Ascis cylindraceutis. Sporidiis ellipticis, binucleatis, hyalinis. Paraphysibus linearibus, supra in processibus brevibus divisis.*”

Although the synonymy between *Pustularia ochracea* and *Tarzetta spurcata* was accepted by authors like NANNFELDT (1938), KORF (1986) or YAO & SPOONER (2002), we believe they must be separated (see our discussion under *T. spurcata*).

This species differs from *Tarzetta catinus* by the absence of a true stipe, although some specimens present a furrowed base, sometimes elongated that resembles a pseudostipe (Pl. 5, fig. C and F), embedded in the substrate. This is a large species reaching 50 mm in diameter and showing a typical ochraceous colour, especially on the oldest specimens. The ascospores measure 20–25 × 11–13 µm after BOUDIER (1904), and 19–24 × 11–13.5 (14) µm after our collections. This species was originally described from the Montmorency forest, in the Paris area, which is mainly composed by chestnut trees (*Castanea sativa*) at 70%, oaks (*Quercus* sp.) at 11% and beech (*Fagus sylvatica*) at 2% [source: ONF]. COOKE (1879) — who figured the species from specimens and drawing sent by Boudier — indicates: “On the ground in beech woods”. Therefore, we hypothesize it may be associated with *Fagaceae*.



Plate 6 – *Tarzetta ochracea*. A: Coll. LY:NV 2018.06.05; photo B. Capoen. B: Coll. LY:NV 2019.06.01 (epitype); photo J.-C. Tinlot. C: Coll. TaCu18061801; photo C. Frund. D: Coll. LY:NV 2018.06.11; photo C. Page. E–F: Coll. M.C. 15-6-20; photos M. Carbone.

We designate an epitype with a recent collection made close to Paris (Pl. 5, fig. B) which fits morphologically well Boudier's concept of this species. Based on our data, the species is widely distributed in Europe.

NANNFELDT (1938) — followed for example by SCHUMACHER (1979) — suggested *Pustularia ochracea* to be a synonym of *Tarzetta catinus*, considering there exists intermediates between typical forms of both species. As the two species share similar habitats, this idea may seem seductive, but it does not reflect the diversity expressed through molecular data.

BRESADOLA (1892) considered *Peziza ochracea* and *P. spurcata* as later synonyms of his *Aleuria pustulata*, based on *Octospora pustulata* Hedw. Although the species described and illustrated by HEDWIG (1789) can figure a *Tarzetta* (see Pl. VI, fig. 1–2, showing pustulate

apothecia, and fig. 5 biguttulate ascospores), it seems difficult to define if this name can apply to *T. ochracea* or not (or to another known species) without authentic material.

Studied material: FRANCE: Essonne, Saint-Pierre-du-Perray, forêt de Rougeau, ~48.587559° N 2.5167034° E, 85 m a.s.l., on soil, under *Quercus* sp. and *Populus tremula*, leg. J.-C. Tinlot, 4 Jun. 2019, herb. N.V. 2019.06.01 (LY), epitype. Doubs, Labergement-Sainte-Marie, in a private garden, 46.775665° N 6.280288° E, 867 m a.s.l., on soil, under *Fagus sylvatica*, leg. C. Page, 5 Jun. 2018, herb. N.V. 2018.06.11 (LY). Labergement-Sainte-Marie, forêt domaniale du Mont Sainte-Marie, near the natural reserve of Remoray, ~46.78233° N 6.261407° E, 960 m a.s.l., in a mixed wood (*Picea abies*, *Fagus sylvatica*), leg. C. Frund, 18 Jun. 2018, pers. herb. TaCu18061801, duplicata N.V.

2018.06.49 (LY). SPAIN: Asturias, Castro, road to Pineda, 43,13624° N 6.26573° W, 665 m a.s.l., on soil, under *Castanea sativa* and *Tilia* sp., leg. N. Van Vooren, 5 Jun. 2013, herb. N.V. 2013.06.12 (LY). SWITZERLAND: Fribourg, Montagny, les Arbognes, forêt de Berley, 46.8124525° N 7.0054741° E, 525 m a.s.l., on soil, under *Fagus sylvatica*, leg. R. Dougoud, 30 Jun. 2013, herb. N.V. 2013.06.43 (LY). Bern, Tramelan, les Embreux, 47.262777° N 7.128583° E, 1085 m a.s.l., on soil, under conifers and *Fagus sylvatica*, leg. B. Capoen, 15 Jun. 2018, herb. N.V. 2018.06.05 (LY). ITALY: Piedmont, Parco natural della Alta Valle Pesio e Tanaro, Pian delle Gorre, 44.2146667° N 7.6608333° E, 1040 m a.s.l., on soil, under *Abies alba* mixed with *Fagus sylvatica*, leg. M. Carbone, 20 Jun. 2015, pers. herb. M.C. 15-6-20 (duplicata in LY). FINLAND: Uusimaa, Helsinki, Länsi-Pakila, ~60.23764° N 24.92213° E, in a garden, on soil, leg. S. Manninen, det. H. Harmaja under *T. spurcata*, 29 Jul. 1970, herb. H6074351.

Tarzetia pusilla Harmaja, *Karstenia*, 14: 116 (1974).

Type: Finland, prov. Kuusamo, Oulanka National Park, the ravine of the brook Tuulilammenpuro, ~66.36407° N 29.444572° E, 20 Aug. 1971, leg. H. Harmaja (H 6074352, holotype) [studied].

Original diagnosis: "A *T. spurcata* (Pers.) Harmaja *apotheciis minoribus et sessilibus, sporis ellipsoides et latioribus et paraphysibus crassioribus, a T. catino* (Pers.) Korf & Rogers *apotheciis minoribus, sporis latioribus et apicibus paraphysium valde ramosis differt.*"

Our revision of the holotype is in conformity with the microscopical description of HARMAJA (1974) except for the size of ascospores which is a bit smaller, \dagger (17.5) 19–21 (22) \times (10.5) 11–12 μm [Qm = 1.8, n=30] vs. 20–23 \times 11.5–13 μm and the rarity of digitate paraphyses (as illustrated by the author on the right part of Fig. 2, top row). We only observed paraphyses with a simple tip or with few bumps, as represented on the left part of Harmaja's fig. 2, top row. We also observed longer ectal hairs, reaching up to 150 μm in length vs. 100 μm in Harmaja's description. Nothing is said about the spore content in the fresh state and it is evident that HARMAJA (*op. cit.*) studied his material from exsiccata, judging by the dead ascospores represented in his Fig. 1a.

Although no associated trees are mentioned in the original description, the habitat "in moist moss on dolomitic rock and stones near the water" and its small size suggest affinities with *Tarzetia alnicola* (this paper) but the latter has larger ascospores (especially the width). Unfortunately, two attempts to obtain rDNA sequences from the type collection failed due to contamination. We regard the Hansen collection KH.03.66 (FH), from Norway, as a reference for this species until a fresh collection from Finland can be examined, sequenced and eventually designated as an epitype.

Tarzetia spurcata (Pers.) Harmaja, *Karstenia*, 14: 119 (1974).

Basionym: *Peziza spurcata* Pers., *Mycologia Europaea*, 1: 226 (1822).

Type: L 0116965, ex herb. Persoon, without mention. This collection was designated as a neotype by HARMAJA (1974) and not holotype, considering that it cannot be proved these specimens were collected before Persoon's publication.

Original diagnosis: "17. *spurcata*, *gregaria majuscula sordide alba; seu subfuliginea, externe farinacea, cupula margine integerrimo. Hab. in sylvis locis adustis, sestate.*"

Sessilis, 1/3 unc. magna, fragilis, grisea, cupulae margo quasi straitus et magis farinaceus, sed non crenatus, uti in sequente, cui haud dissimilis."

As the neotype of *Peziza spurcata* is part of Persoon's historical herbarium and unavailable for DNA extraction, we morphologically and molecularly investigated one of the studied collections cited by HARMAJA (1974) when he combined this name within *Tarzetia* (coll. H 6074351). Our revision of the latter gives the following microscopical characters (in water): **Medullary excipulum** of *textura intricata*, with yellowish hyphae. **Ectal excipulum** thin, of *textura angularis*, with cells up to 48 μm wide; presence of emerging septate hyphae, hyaline, reaching 170 μm in length and 3–5 μm wide. **Paraphyses** not enlarged at the top, \pm diverticulate. **Ascospores** ellipsoid, with tapered ends, \dagger (19.5) 20–23 (23.5) \times 11–13 μm , Qm = 1.8 (n=30), hyaline, smooth, biguttulate.

Unfortunately, our attempt to extract DNA from this sample failed, but we believe that this collection refers to *T. ochracea* (see under this name).

Contrary to the opinion of HARMAJA (1974) who suggests that "the younger *Peziza ochracea* Boud. in Cooke is almost surely a synonym", we do not believe this hypothesis. The Finnish author was probably influenced by Boudier's drawing of paraphyses (BOUDIER, 1905-1910, pl. 337, fig. f), partly similar to the ones he depicted in fig. 2, lower row (HARMAJA, *op. cit.*). But BOUDIER (*op. cit.*: 188) writes: "Paraphyses [...] à peine épaissies au sommet qui est souvent simple, mais quelquefois divisé en deux ou trois petits rameaux courts." which means "Paraphyses [...] barely thickened at the top which is often simple, but sometimes divided into two or three small and short branchlets." Harmaja also describes a large species, reaching 45 mm in diameter. This is the case for *Pustularia ochracea* — Boudier gives a range between 20 to 50 mm — but not for *Peziza spurcata* in its original sense, PERSOON (1822) indicating about 8–9 mm in diameter (confirmed by the dried specimens of neotype, L 0116965, see plate 7). The colour given by PERSOON (*op. cit.*) seems also different, "*sordide alba... grisea*" versus pale ochraceous in *P. ochracea* after Boudier's description (see also the plate 337). At last *P. spurcata* is described as growing on burnt ground in a forest during summer ("*in sylvis*

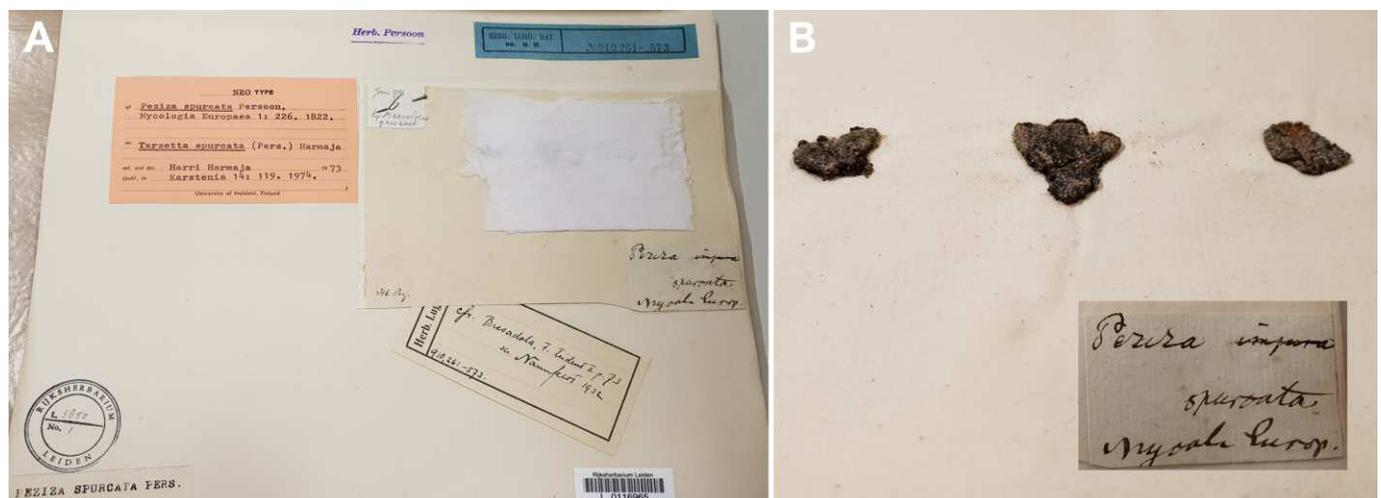


Plate 7 – *Peziza spurcata*. A: Voucher of the authentic material of *Peziza spurcata* Pers. B: Detail of the specimens. Photos Roxali Bijmoer (Naturalis Biodiversity Center, Leiden).

locis adustis, aestate”). We think that HARMAJA (1974) misinterpreted *Peziza spurcata*, which must be considered different from *Pustularia ochracea* Boud. For these reasons and the impossibility to trace the original locality of *Peziza spurcata* we consider this name as a *nomen ambiguum*.

Tarzetta velata (Quél.) Svrček, *Česká Mykol.*, 35 (2): 88 (1981).

Basionym: *Peziza velata* Quél., *C. R. Assoc. fr. Avanc. Sci.*, 24 (2): 621 (1896).

Type: no type material exists.

Lectotype here designated: Plate VI, fig. 17, QUÉLET (1896); MBT 389446.

Original diagnosis: “Peridium cupulaire (0m,01), stipité, avec la marge crénelée, fragile, translucide, blanc crème grisonnant, couvert de grains pulvérulents brunâtres. Hyménium crème, fermé par un voile soyeux et blanc qui s’ouvre en étoile par le développement de la cupule. Spore ellipsoïde (0mm,015), biguttulée, hyaline. (Pl. VI, fig. 17.)

Été. — Sur l’humus formé par les souches de hêtre, Jura. Cette jolie *peziza* ne paraît pas spécifiquement différente de *cupularis* et pourrait être rapportée à *catinus*.”

Homotypic synonyms: *Geopyxis velata* (Quél.) Sacc. & P. Syd., *Syll. fung.*, 14: 744 (1899); *Pustularia catinus* var. *velata* (Quél.) Boud., *Hist.*

class. Disc. Eur.: 53 (1907); *Pustularia velata* (Quél.) Le Gal, *Bull. Soc. mycol. France*, 70 (3): 200 (1955); *Pustulina velata* (Quél.) Svrček, *Česká Mykol.*, 31 (2): 70 (1977); *Tarzetta cupularis* var. *velata* (Quél.) Häffner, *Rheinl.-Pfälz. Pilz.*, 2 (1): 43 (1992).

Taxonomical synonyms: *Geopyxis alba* Velen., *Novit. Mycol. Novissim.*: 152 (1947), *fide* SVRČEK (1979); *Geopyxis radicans* Velen., *Monog. Disc. Bohem.*: 337 (1934), *fide* SVRČEK (1979).

The abundant veil at the margin is the main feature that justified the creation of this taxon by QUÉLET (1896), but several species of *Tarzetta* present such a character in the young state (e.g. *Tarzetta al-nicola* – see under this name), so this cannot be considered as taxonomically valuable.

The main work done on this species came from LE GAL (1955) who recognized *T. velata* in a collection made in Switzerland in a beech forest. Although she gave a detailed description and illustrations, it is hard to determine which species could be applied to her collection. An examination of this collection with our modern techniques is required to fix this name, among the taxa known to be associated with *Fagaceae*. The synonyms proposed by SVRČEK (1979) — based on Velenovský’s collections — also require investigation in the light of our results and the heterogeneity of spore size given by the Czech author (see Table 2).

Table 2 – Comparison of some characters of *T. velata* in the literature

Author	Apothecia size (in mm)	Spore size (in µm)
QUÉLET (1896)	10	15
LE GAL (1955)	up to 8 mm	(17.25) 19–22 × (11) 12–14
SVRČEK (1979), sub <i>Geopyxis alba</i>	10	19–22 × 11.5–13
SVRČEK (1979), sub <i>Geopyxis radicans</i>	7–15	20–22–25.5 × 12–14–15.5
HÄFFNER (1992), sub <i>T. cupularis</i> var. <i>velata</i>	up to 27.5 mm	18–22 × 11–14

Tarzetta jafneospora W.Y. Zhuang & Korf, *Mycotaxon*, 28 (2): 365 (1987). Plate 8 and fig. 4.

Type: New Zealand, Gisborne, Urewera National Park – PDD 49572 (holotype); CUP 61778 (isotype).

Original diagnosis: “*Ab Tarzetta speciebus aliis ascosporis fusoides cum ornamentation cyanophilica differens.*”

This species is only known from New Zealand (ZHUANG & KORF, 1987) where it is considered as a rather common species, probably

associated with *Nothofagus* trees. It occupied an isolated position in our phylogenetic tree of LSU sequences (Fig. 1). Morphologically it possesses warted ascospores, a character unique in the genus. The elongated fusoid shape of ascospores is also characteristic.

Macroscopically it can be confused with *Jafnea pallida* (Rodway) G.M. Gates & Van Vooren, a species known from Tasmania, also having fusoid and warted ascospores but of different type and asco-carps being stipitate (GATES & VAN VOOREN, 2016).



Plate 8 – *Tarzetta jafneospora*. A: Coll. PDD 96264; photo J.A. Cooper (Landcare Research). B: Coll. CSAK 416; photo C. Shirley.

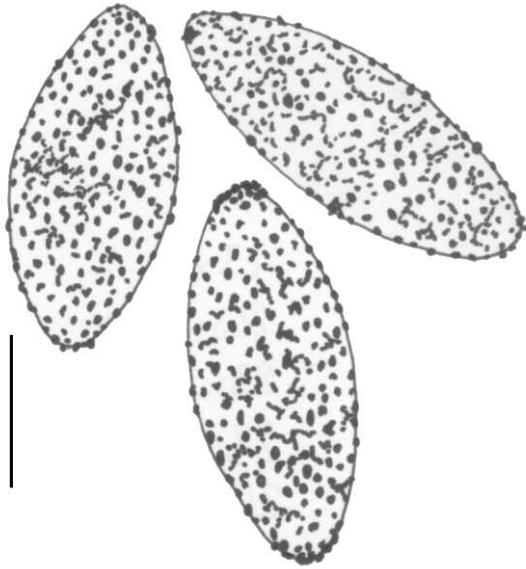


Fig. 4 – Ascospores of *Tarzetta jafneospora* in Cotton Blue (coll. CSAK 416). Scale bar = 10 μ m. Drawing N. Van Vooren

Apothecia 5–8 mm diam., sessile, cupuliform, with a greyish to livid grey hymenium; external surface beige or brownish grey, pustulate. **Margin** denticulate.

Medullary excipulum of *textura intricata*, with hyaline hyphae. **Ectal excipulum** rather thin, of *textura suglobulosa/angularis*, with hyaline cells, \dagger 10–35 μ m, forming in the outer part some pyramidal

cells aggregates. **Marginal cells** of *textura prismatica*. **Asci** \dagger 300–320 \times 12–14 μ m, cylindrical, narrowed to the base, without crozier, operculate, inamyloid, 8-spored. **Paraphyses** filiform, \dagger 2.5–3 μ m diam., not enlarged at the top, straight, simple, hyaline. **Ascospores** \dagger (20.5) 23–26 (27) \times (8) 10–11.5 μ m [$X = 24.6 \times 10.6 \mu$ m, $n=30$], $Q=2.1-2.7$, $Q_m=2.3$, fusoid, hyaline, rather thick-walled, containing two large oil drops, ornamented with cyanophilic low warts, dense, isolated or coalescent, \pm angular, up to 0.5 μ m wide, often concentrated at the poles and looking like caps.

Studied material: NEW ZEALAND: Auckland district, Hunua, Workman Road, Workman Track, $\sim 37.122333^\circ$ S 175.233742 E, on mossy soil, in a *Leptospermum* and *Nothofagus* mixed forest, 14 Jun. 2015, leg. C. Shirley, det. N. Van Vooren, duplicata of pers. herb. CSAK 416.

Tarzetta bronca (Peck) Korf & J.K. Rogers, in Korf, *Phytologia*, 21 (4): 206 (1971). Plate 9 and fig. 5F.

Basionym: *Peziza bronca* Peck, *Ann. Rep. N.Y. St. Mus. Nat. Hist.*, 29: 54 (1878).

Type: USA, New York State – NYS F-000544 (syntype).

Original diagnosis: “Cups gregarious or crowded, sessile, sub-hemispherical, four to nine lines broad, whitish or very pale-buff, externally roughened by small crowded whitish warts; asci cylindrical; spores elliptical, one to two-nucleate, .0008-.0009' long, .0005' broad.

Ground. Knowersville and Sandlake. July and August. (Plate 2, figs. 10-12.)”

Homotypic synonyms: *Geopyxis bronca* (Peck) Seaver, *N. Amer. Cup-fungi (Operculates)*: 213 (1928); *Pustularia bronca* (Peck) Kanouse, *Mycologia*, 42 (4): 497 (1950); *Pustulina bronca* (Peck) Korf & Berthet, *Natural. Can.*, 96 (2): 248 (1969).



Plate 9 – *Tarzetta bronca*. Coll. SAK-17-0423-5; photo S. Krstic.

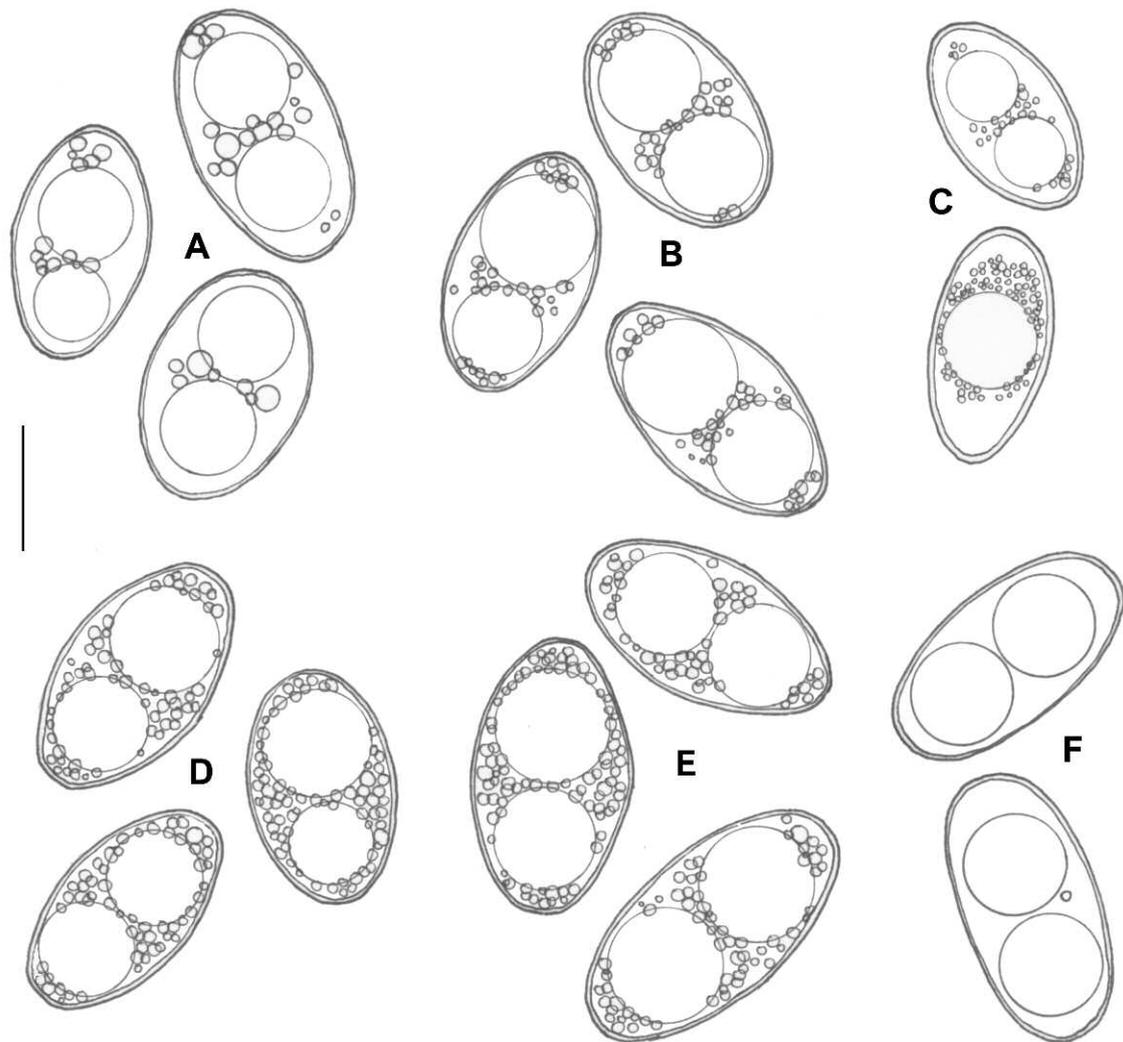


Fig. 5 – Ascospores of *Tarzetta* species. A: *T. catinus* (coll. LY:NV 207.04.18). B: *T. cupularis* (coll. LY:NV 2019.05.11). C: *T. betulicola ad int.* (coll. M.P. 2018-133). D: *T. pseudocatinus* agg. (coll. LY:NV 2019.05.12). E: *T. ochracea* (coll. LY:NV 2019.06.01, epitype). F: *T. bronca* (coll. SAK-17-0423-5). Scale bar = 10 μm . Drawings N. Van Vooren.

Shortly described by PECK (1878) from the state of New York, the species was redescribed by KANOUSE (1950) based on numerous collections coming from different North American localities. KANOUSE (*op. cit.*), in her microscopical description, gave two different sizes of asci and ascospores, and evoked that “heterosporous condition was found in all of the 29 collections” and also in the type collection.

Macroscopically the species seems characterized by its yellowish tinges of ascocarps, especially visible on outer surface of dried specimens (KANOUSE, 1950: 499) but also on fresh specimens (SEEVER, 1928: 213). We selected a collection that may represent *T. bronca*. We measured ascospores \dagger (19) $20.5\text{--}22 \times (10) 10.5\text{--}12 \mu\text{m}$ [$X = 21.2 \times 11.2 \mu\text{m}$, $Q_m = 1.9$, $n = 30$] vs. $20\text{--}24 \times 12\text{--}14 \mu\text{m}$ in KANOUSE (*op. cit.*) and also noted the presence of numerous immature asci (so with smaller ascospores) that may correspond to the “heterosporous condition” indicated by Kanouse.

The taxonomic diversity of *Tarzetta* in North America requires exploration in the light of this preliminary work.

Studied material: USA, Oregon, Multnomah Co., Portland, Kelley Point Park, $\sim 45.6463889^\circ \text{N } 122.7625^\circ \text{W}$, 35 m a.s.l., on soil, under cottonwoods (*Populus* sp.), leg. S. Krstic, 23 Apr. 2017, duplicata herb. SAK-17-0423-5 (as *T. cupularis*).

Taxonomy

Following the results of molecular, morphological and ecological data, we describe and illustrate here six new species, and propose another one *ad interim*.

Tarzetta gregaria Van Vooren, *sp. nov.* – MB 833286 – Pl. 10

Diagnosis: Differs from the other *Tarzetta* spp. by its gregarious habit of growth, its orangish colour, and its genetic profile.

Holotype: France, coll. NV 2017.08.16 (LY).

Etymology: From Latin “gregarius” which means “related to the herds”, as in very numerous.

Ascomata gregarious. **Apothecia** 1–3 mm diam., sessile, cupuliform, with a beige orangish hymenium; external surface concolorous, finely white-powdery. **Margin** eroded. **Flesh** thin.

Subhymenium and **medullary excipulum** of *textura intricata*, with hyaline hyphae. **Ectal excipulum** of *textura globulosa/subglobulosa*, with cells 10–32 μm diam., mixed with some clavate elements.

Marginal cells of *textura subprismatica*, composed of hyaline hyphae, 4–7 μm wide, with their top slightly emerging. **Asci** 310–360 \times 15–17 μm , cylindrical, long narrowed to the base, without crozier, operculate, inamyloid, 8-spored. **Paraphyses** filiform, 3–3.5 μm diam., not enlarged at the top, straight or slightly curved at the top, simple, hyaline. **Ascospores** (20) $21\text{--}24.5$ (25.5) \times (11.5) 12.5–15 μm

[X = 21 × 13 μm, n=30], Q=1.1–1.3, Qm=1.2, ellipsoid, sometimes slightly tapered at the ends or subfusoid, hyaline, smooth, rather thin-walled, containing two large oil drops, rarely only one, with numerous small guttules.

Studied material: FRANCE: Savoie, Beaufort, lac de la Gittaz, above the dam, 45.71546° N 6.66664° E, 1675 m a.s.l., on soil, in the border of a wood of green alders (*Alnus alnobetula*), leg. N. Robert, 29 Aug.

2017, herb. LY NV 2017.08.16 (holotype) and pers. herb. B. Capoen BC250817 (isotype).

Comments: The great number of apothecia (about one hundred specimens on a small surface) with a light orangish colour was reminiscent of a *Sepultariella* species (VAN VOOREN *et al.*, 2017), but the examination of microscopical characters changed this initial super-

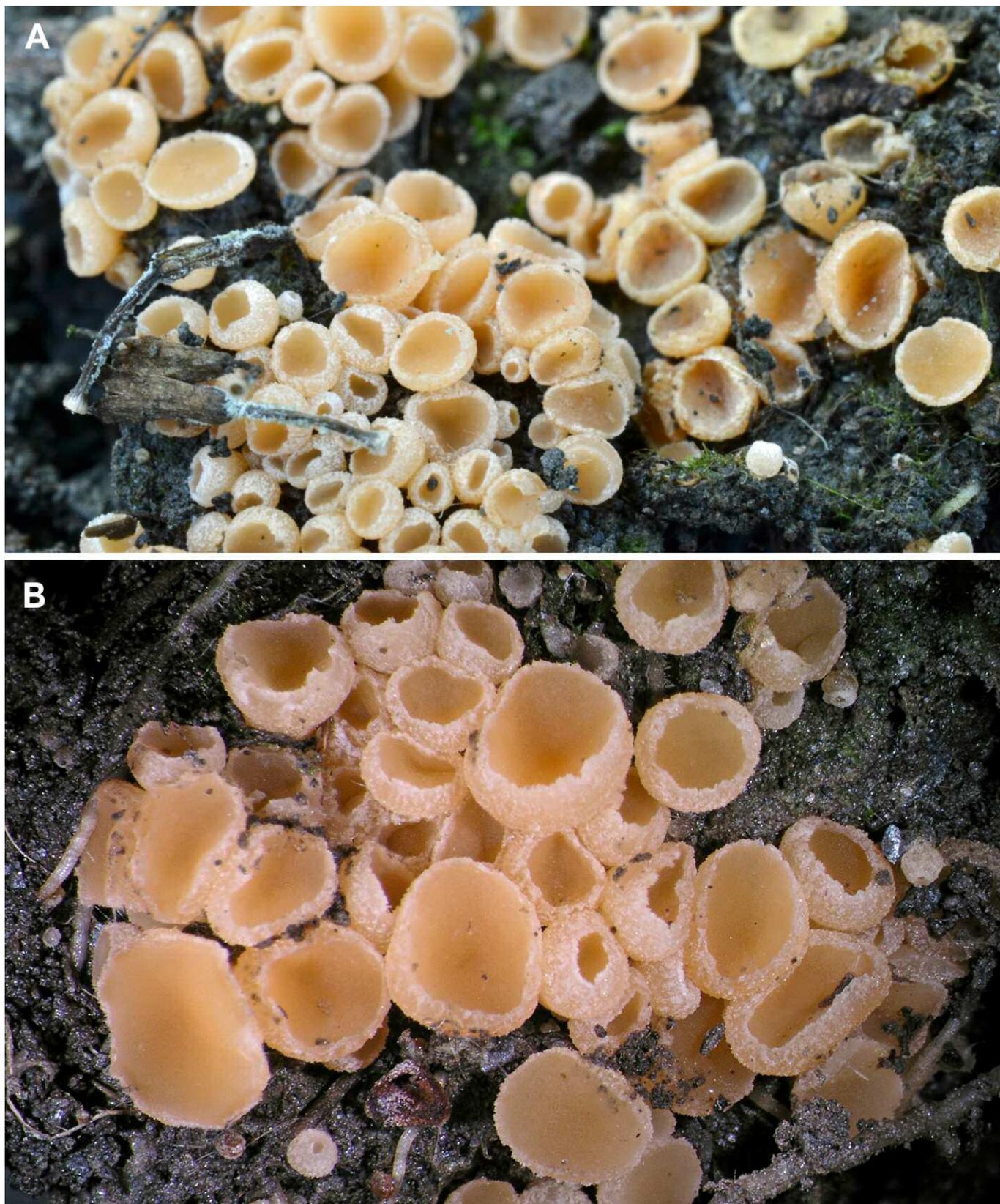


Plate 10 – *Tarzetta gregaria*. A: Coll. LY:NV 2017.08.16 (holotype); photo N. Van Vooren. B: idem; photo R. Dougoud.

ficial identification. The phylogenetic analyses (Fig. 1 & 2) confirmed the placement in *Tarzetia* in an isolated position. Its specific characters and the molecular data justify its publication as a new species.

ITS and LSU sequences of *T. gregaria* are identical to an environmental sequence isolated from soil in the Arctic part of Canada (GenBank KC966365), in a site characterized by “prostrate dwarf shrub, herb tundra (*Dryas integrifolia*-*Salix arctica*)” (TIMLING *et al.*, 2014). Since no *Dryas* sp. or dwarf willows were growing in the site where *T. gregaria* was collected, we speculate that it could be associated with another boreo-alpine plant.

Tarzetia sepultarioides Van Vooren, *sp. nov.* – MB 833297 – Pl. 11

Diagnosis: Differs from other sessile *Tarzetia* spp. by its ascus base often enlarged or with a protuberance (as if it was pleurorhynchous).

Holotype: France, coll. NV 2017.08.03 (LY).

Etymology: From “*Sepultaria*”, the genus name in which I first placed the type collection, and *-oides* meaning “resembling”.

Apothecia 4–6 mm diam., sessile, cupuliform, with a pale beige to pale greyish hymenium; external surface finely furfuraceous or low-warted, pale beige to light ochraceous. **Margin** eroded or ± postulate, concolorous or ochraceous.

Medullary excipulum of *textura intricata*, with hyaline hyphae.

Ectal excipulum of *textura subglobulosa/angularis*, with cells 10–35 µm diam., hyaline to light yellow, ± organized in small pyramidal brownish warts; presence of some emerging hyphae, septate, rather short, 40–155 (260) × 7–8 µm. **Asci** 260–280 × 18–21 µm, cylindrical, long narrowed to the base, without crozier but often enlarged (as if they were pleurorhynchous), operculate, inamyloid, 8-spored. **Paraphyses** filiform, 5–8 µm diam., not enlarged at the top, sometimes sublanceolate, simple, hyaline. **Ascospores** 22–25 (26.5) × (11.5) 12–13 µm [$X = 23.9 \times 12.4 \mu\text{m}$, $n=20$], $Q=1.8\text{--}2.1$, $Q_m=1.9$, ellipsoid tapered at the ends to fusoid, hyaline, smooth, thick-walled, containing two large oil drops, with small guttules, the latter lacking in mature spores.

Studied material: FRANCE: Savoie, Beaufort, la Tetaz, 45.7073° N 6.61507° E, 1000 m a.s.l., along the river Doron, on wet naked soil,

under *Petasites* sp., in a riparian woodland (*Alnus incana*, *Acer* sp. and *Picea abies*), leg. N. Van Vooren, 27 Aug. 2017, herb. NV 2017.08.03 (LY), holotype.

Comments: On the field, *Tarzetia sepultarioides* was first “determined” as a *Sepultaria* species. A first microscopical examination confirmed partially this feeling because of the ascospores and the enlarged ascus base, but at the same time, the absence of true hairs emerging from the ectal excipulum and an outer surface marked with clustered cells excluded this hypothesis. The rDNA analysis of the collection demonstrated this species was a member of *Tarzetia*.

Based on its LSU sequence our collection is 99% identical to a Danish collection (C:F-55462) identified as *T. gaillardiana*. As described by BOUDIER (1902; 1905-1910), the microscopical characters of *T. sepultarioides* seem very close, but as we discussed above, the review of the isotype of *T. gaillardiana* is not fully in conformity with the protologue (especially the spore size). Moreover the species depicted by BOUDIER (*op. cit.*) is shortly stipitate and mature ascospores content is different. For these reasons we do not believe that our collection is *T. gaillardiana*.

Tarzetia alnicola Van Vooren, *sp. nov.* – MB 833287 – Pl. 12

Diagnosis: Differs from other *Tarzetia* spp. by its small size, association with a habitat in riparian woodland, under alder trees (*Alnus* spp.), and its genetic profile.

Holotype: France, coll. NV 2017.08.36 (LY).

Etymology: From Latin “*alnus*”, meaning alder, and “*cola*” meaning “linked to”.

Misapplication: *Tarzetia velata* in VAN VOOREN (2017).

Apothecia 2.5–9 (18) mm diam., sessile or shortly stipitate, cupuliform, with a whitish to pale cream hymenium; external surface beige to buff, finely pustulate with scattered small reddish-brown tufts. **Margin** denticulate and a bit darker than the outer surface, with an abundant veil in young state. **Flesh** thin. **Stipe** buried in the substrate, sometimes with a white mycelial tomentum.

Subhymenium and **medullary excipulum** thick, of *textura intricata*, with hyaline hyphae. **Ectal excipulum** of *textura globulosa/subglobulosa*, with cells 7–30 µm diam., mixed with some clavate el-



Plate 11 – *Tarzetia sepultarioides*. A: Coll. LY:NV 2017.08.03 (holotype). B: Ascus base in CR. All photos N. Van Vooren.

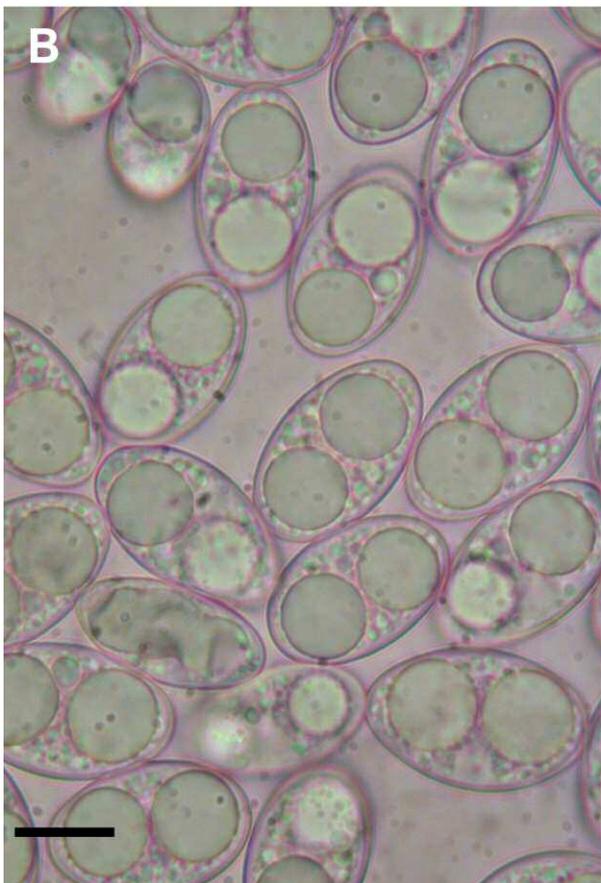


Plate 12 – *Tarzettia alnicola*. A: Coll. LY:NV 2017.08.36 (holotype). B: Ascospores in living state, in water (scale bar = 10 μ m). C: Coll. LY:NV 2017.08.33. D: Coll. LY:NV 2013.08.26. All photos N. Van Vooren.

elements, up to 65 µm wide, forming in the outer part some cells aggregates, brownish-coloured. **Marginal cells** of *textura subprismatica*, composed of hyaline hyphae, 6–13 µm wide, with some marginal “hairs”, 120–180 × 6–12 µm, composed of cell chains, pale ochre. **Asci** (250) 280–340 × 14–17 (20) µm, cylindrical, long narrowed to the base, without crozier, operculate, inamyloid, 8-spored. **Paraphyses** filiform, 3–4 (6) µm diam., straight or slightly enlarged at the top, sometimes sublanceolate, more rarely subclavate, simple, hyaline. **Ascospores** (20) 21–24.5 (25.5) × (12) 13–15 µm [$X = 22.8 \times 13.9 \mu\text{m}$, $n=108$], $Q=1.5\text{--}1.8$, $Q_m=1.6$, widely ellipsoid, slightly tapered at the ends, hyaline, smooth, rather thick-walled, containing two large oil drops, and numerous small guttules which fill all the space, especially in asci.

Studied material: FRANCE: Savoie, Hauteluce, l’Infernet, 45.76657° N 6.62345° E, 1160 m a.s.l., on wet soil, along the brook Dorinet, under *Petasites hybridus*, *Alnus* sp. and *Fraxinus excelsior*, 30 Aug. 2017, leg. N. Van Vooren, NV 2017.08.33 (LY). Savoie, Beaufort, la Tetaz, 45.7073° N 6.61507° E, 1000 m a.s.l., along the river Doron, on wet naked soil, under *Alnus incana*, *Acer* sp. and *Picea abies*, 31 Aug. 2017, leg. N. Van Vooren, NV 2017.08.36 (LY) [holotype]; same place, 28 Aug. 2013, leg. N. Van Vooren, NV 2013.08.26 (LY). Savoie, Beaufort, près du lac de la Gittaz, 45.719212° N 6.667183° E, 1620 m a.s.l., along a rivulet, with *Alnus alnobetula* in the vicinity, 24 Aug. 2013, leg. B. Jeannerot, NV 2013.08.06 (LY). Alpes-de-Haute-Provence, Castellane, near the camping “la Ferme”, 43.8376° N 6.54244° E, 760 m a.s.l., along the brook of Destourbes, on wet naked soil, under *Alnus* sp., 9 Sept. 2017, leg. M. Vega & N. Van Vooren, NV 2017.09.08 (LY). Rhône, La Tour-de-Salvagny, Cerqueminal, 45.808192° N 4.699982° E, 280 m a.s.l., along the brook of “la Grande Rivière”, on naked soil, under *Alnus glutinosa* and *Fraxinus excelsior*, 14 Jul. 2008, leg. N. Van Vooren, NV 2008.07.06 (LY).

Comments: Initially we used the name *T. velata* for these collections made along small rivers (VAN VOOREN, 2017), partly based on the concept of HÄFFNER (1992). The DNA analyses revealed the originality of this taxon, inside the /tarz-8 clade (Fig. 1 and 2), based on its habitat and association with alder trees, confirmed by environmental ITS sequences present in public databases (e.g. GenBank

KX897769, with *Alnus glutinosa* subsp. *barbata*; FM993170, with *Alnus* sp.).

Macroscopically it is characterized by small apothecia, less than 10 mm diam. in our collections (up to 18 mm in one specimen), and an outer surface finely marked by darker pustules. Microscopically, it is characterized by its wide ascospores, fully filled by the two oils drops and small guttules.

Tarzettia alpina Van Vooren & Cheype, *sp. nov.* – MB 833290 – Pl. 13

Diagnosis: Differs from other *Tarzettia* spp. by its non-pustulate outer surface, becoming dark brown at the margin, its boreo-alpine habitat and its genetic profile.

Holotype: France, coll. NV 2009.08.11 (LY).

Etymology: From Latin “*alpinus*”, the Alps, referring to its growth at the alpine zone.

Apothecia 5–11 mm diam., sessile or substipitate, deeply cupuliform, with a beige to yellow beige hymenium, becoming darker with age; external surface finely furfuraceous but not pustulate, beige, caramel, becoming dark brown near the margin. **Margin** whitish, teeth-like when young, then eroded or jagged.

Medullary excipulum of *textura intricata*, with hyaline hyphae. **Ectal excipulum** mainly of *textura globulosa*, with cells hyaline to yellowish, mixed with some clavate or subglobose elements. **Asci** 250–300 × 14–16 µm, cylindrical, long narrowed to the base, without crozier, operculate, inamyloid, 8-spored. **Paraphyses** filiform, 4–6 µm diam., not enlarged at the top, straight, simple or forked at the middle, rarely diverticulate, hyaline. **Ascospores** 20.5–23 × 11–13.5 (14) µm [$X = 22.1 \times 12.5 \mu\text{m}$, $n=21$], $Q=(1.5) 1.6\text{--}2.0$, $Q_m=1.8$, ellipsoid, tapered at the ends to subfusoid, hyaline, smooth, rather thin-walled, containing two large oil drops, with numerous small guttules.

Studied material: FRANCE: Savoie, Aime-la-Plagne, Cormet d’Arêches, 45.617421° N 6.608059° E, 2100 m a.s.l., on wet soil, among alpine plants, leg. J.-L. Cheype, 27 Aug. 2009, herb. NV 2009.08.11 (LY, holotype) and pers. herb. JLC (isotype).



Plate 13 – *Tarzettia alpina*. A: Coll. LY:NV 2009.08.11 (holotype); photo J.-L. Cheype. B: Ascospores in living state, in water (scale bar = 10 µm); photo N. Van Vooren.

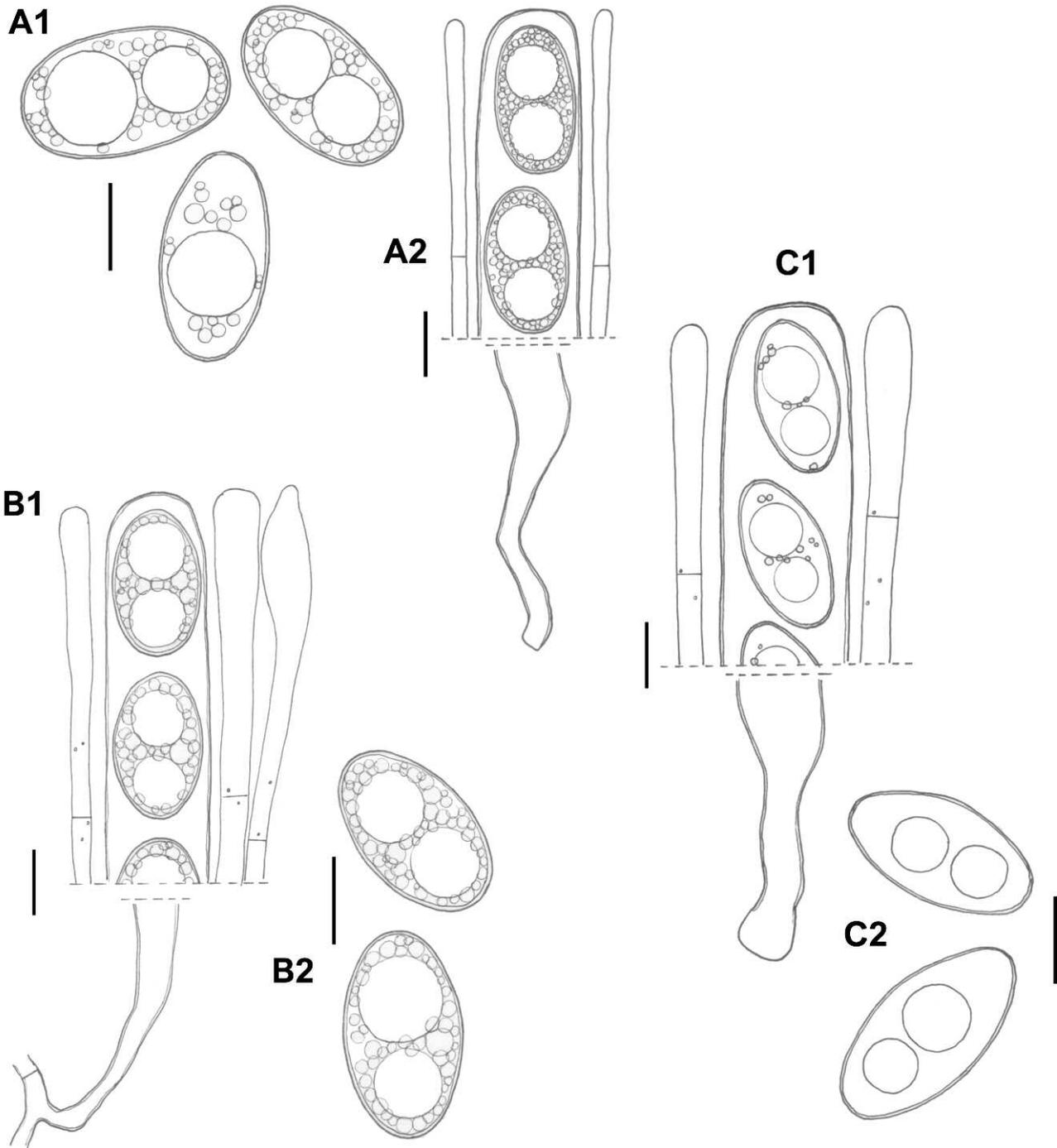


Fig. 6 – Microscopic characters. **A:** *Tarzetta gregaria* (coll. LY:NV 2017.08.16, holotype); 1: Ascospores; 2: Paraphyses and ascus. **B:** *Tarzetta alnicola* (coll. LY:NV 2013.08.26); 1: Ascospores; 2: Paraphyses and ascus. **C:** *Tarzetta sepultarioides* (coll. LY:NV 2017.08.03, holotype); 1: Paraphyses and ascus; 2: Ascospores. Scale bars = 10 µm. Drawings N. Van Vooren.

Comments: In the field, *Tarzetta alpina* can easily be confused with a *Sepultaria* species. This impression is consolidated by the sessile ascomata, the absence of a pustulate outer surface and the general colour. A microscopical examination — especially on fresh material — allows observation of the usual features of a *Tarzetta*. We are not able to determine the plant association and new collections are required to propose hypotheses on this ecological character.

T. alpina is phylogenetically identical to an unnamed Norwegian collection (FH:KH.03.102) but we could not obtain information on the latter to compare its characters and ecology with our own collection. Another collection from Norway, sharing a similar habitat, collected by one of us (M. Carbone) — tentatively named 'cf. *gaillar-*

diana' — was examined. Macroscopically it differs by the outer surface covered by numerous white pustules. Microscopically it also showed some differences: smaller ascospores, (18) 19–20.5 × 10–11 µm, and paraphyses strongly digitate. Unfortunately, our attempt to extract DNA failed. Finally, another collection — from Sardinia (Italy) — is nested in the same clade of *T. alpina* (Fig. 1). The ascospores measure 21–25 × 12–13.5 µm [$X = 23 \times 12.8 \mu\text{m}$, $n=22$], $Q_m=1.8$, on rehydrated material. We hesitate to place it under the same epithet because we do not have any information on its ecology (and biotopes in Sardinia are very different from those of boreo-alpine regions). More genes are required to better evaluate its taxonomical position.

Tarzetta melitensis Sammut & Van Vooren, *sp. nov.* – MB 833298 – Pl. 14

Diagnosis: Differs from *Tarzetta cupularis* and *T. quercus-ilicis* by longer ascospores, a different spore shape and its genetic profile.

Holotype: Malta, coll. CS871 (LY).

Etymology: From “*Melita*”, the Latin name of the island of Malta where the species was collected.

Apothecia 7–11 mm diam., sessile or shortly stipitate, deeply cupuliform, goblet-shaped; hymenium cream-whitish; external surface pustulate, grey-brown, a bit more beige near the margin, with paler pustules. **Margin** distinctly crenate, with reddish brown tooth.

Medullary excipulum of *textura intricata*, ~350–450 µm thick, with hyaline hyphae, 3.5–7 µm diam. **Ectal excipulum** of *textura angularis/subglobulosa*, ~100–150 µm thick, with cells 11–30 × 12–20 µm, and aggregates organized into pyramidal warts, pale orange-brown. **External hyphae** scattered, 4.5–7 µm diam., originating from the ectal excipulum, simple or branched, thin-walled, septate, hyaline. **Asci** 280–325 × 14–19 µm, cylindrical, long narrowed to the base, without crozier, operculate, inamyloid, 8-spored. **Paraphyses** filiform, slightly inflated at the top, 3–7 µm diam., straight, simple or occasionally forked at the base, hyaline. **Ascospores** (21.3) 22.9–26.5 × 12.2–13.4 µm [$X = 24.6 \times 12.9 \mu\text{m}$, $n=45$], $Q = 1.7\text{--}2.0$, $Q_m = 1.9$, ellipsoid with tapered ends to subfusoid, hyaline, smooth, rather thick-walled, containing two large oil drops, with numerous small guttules.

Studied material: MALTA: Buskett, 35.856111° N 14.398778° E, 200 m a.s.l., on soil, under broadleaved trees, one specimen, *leg.*

C. Sammut, 18 Apr. 2012, pers. herb. CS368. Same location, close to *Quercus ilex* and *Laurus nobilis*, several scattered specimens, 18 Apr. 2015, *leg.* C. Sammut, herb. CS871 (LY, holotype).

Comments: Initially determined as *T. gaillardiana* due to its small size and colour, the molecular data confirmed the originality of this taxon. Based on our revision of type-material of *T. gaillardiana*, *T. melitensis* has longer ascospores, 22.9–26.5 × 12.2–13.4 µm vs. 21–24 × (11) 11.5–14 µm.

The closest ITS sequence comes from an EcM isolate (GenBank JF927143, 97.8% similar) from roots of *Quercus pubescens* in a *Tuber melanosporum* truffle ground in Italy (BELFIORI *et al.*, 2012). We hypothesize that *T. melitensis* makes ectomycorrhizae with Mediterranean oak trees like *Q. ilex*, *Q. pubescens*, etc. and being endemic to Malta.

Tarzetta quercus-ilicis Van Vooren & M. Carbone, *sp. nov.* – MB 833292 – Pl. 15

Diagnosis: Differs from the *Tarzetta cupularis* by its sessile ascocarp, its greyish hymenium, its probable strict association with *Quercus ilex* and its genetic profile.

Holotype: France, coll. NV 2014.03.20 (LY).

Etymology: From “*Quercus ilex*”, the Latin botanical name of the host tree.

Apothecia 3–15 mm diam., sessile, deeply cupuliform, spreading at maturity; hymenium pale grey, a bit darker with age; external surface postulate, especially at the margin, ochraceous grey. **Margin**

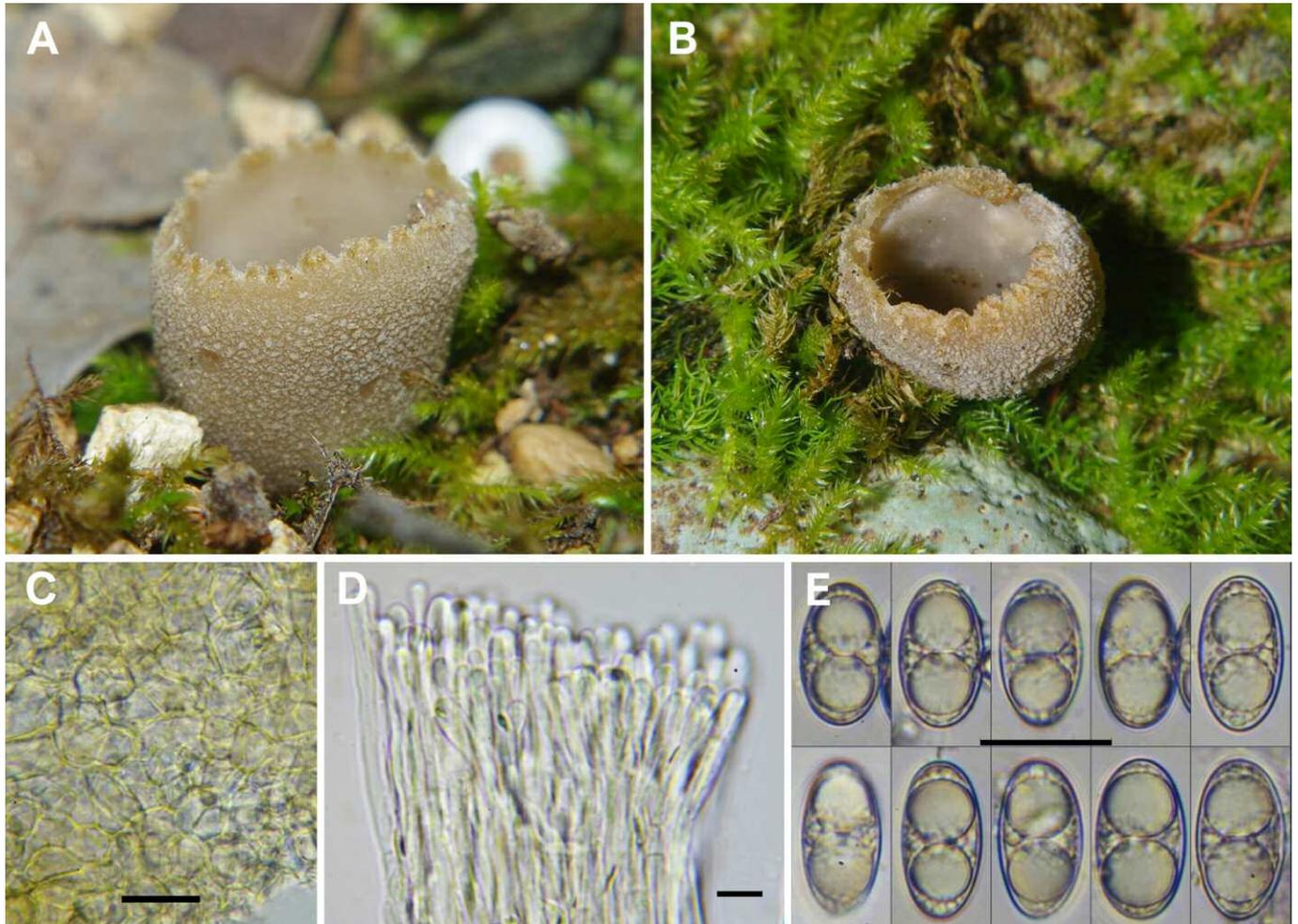


Plate 14 – *Tarzetta melitensis*. A–B: Coll. LY:CS871 (holotype). C: Cells of ectal excipulum, in water (scale bar = 25 µm). D: Top of paraphyses, in water (scale bar = 10 µm). E: Ascospores in living state, in water (scale bar = 20 µm). All photos C. Sammut.

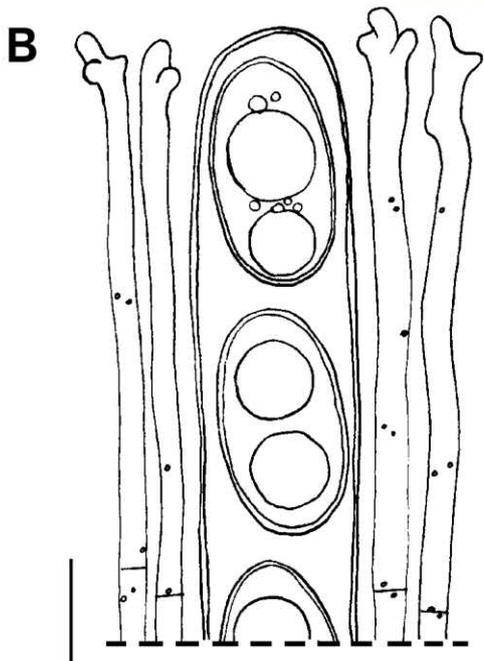


Plate 15 – *Tarzetta quercus-ilicis*. A: Coll. LY:NV 2014.03.20 (holotype); photo N. Van Vooren. B: Top of ascus and paraphyses (scale bar = 10 μ m), drawn from the holotype. C: Coll. M.C. 14-3-15; photo M. Carbone.

eroded or denticulate, concolorous or darker but often with whitish meshes.

Medullary excipulum of *textura intricata*, with hyaline hyphae. **Ectal excipulum** of *textura globulosa/angularis*, with cells 10–30 µm diam. or 18–60 × 11–23 µm. **Asci** 250–300 × 13–18 µm, cylindrical, long narrowed to the base, without crozier, operculate, inamyloid, 8-spored. **Paraphyses** filiform, 4–5 µm diam., straight, hyaline, not enlarged but irregular at the top, ± digitate or with lateral bumps. **Ascospores** 20–23 (24) × (11.5) 12–13 µm [$X = 21.7 \times 12.1$ µm, $n=30$], $Q=1.6-2.0$, $Q_m=1.8$, † 20–22 (23) × 11–12.5 (13) µm [$X = 21.2 \times 11.9$ µm, $n=20$], $Q=1.7-1.9$, $Q_m=1.8$, ellipsoid, sometimes a bit tapered at the poles, hyaline, smooth, thick-walled, containing two large oil drops, sometimes with small guttules.

Studied material: FRANCE: Var, La Seyne-sur-Mer, Jarnas forest, 43.07192° N 5.8536568° E, 77 m a.s.l., on soil, under *Quercus ilex*, leg. F. Fouchier & N. Van Vooren, 22 Mar. 2014, herb. NV 2014.03.20 (LY, holotype). ITALY: Apulia, Brindisi, Tukuran, Bosco Colemi, 40.5362222° N 17.93875° E, 50 m a.s.l., on soil, under *Quercus ilex*, leg. M. Carbone & C. Agnello, 15 Mar. 2014, pers. herb. M.C. 14-3-15 (duplicata in LY).

Comments: This species belongs to a small clade, named /tarz-5b (Fig. 1 and 2), containing taxa from the Mediterranean area. The second taxon was growing in an urban park containing typical Mediterranean vegetation and trees (coll. LY:NV 2001.03.01, France, Fréjus, March 2001). Its LSU sequence is close or identical to an environmental sequence (partial LSU) extracted from roots of *Pinus pinaster* in Spain (GenBank JQ976016). Taking account of the description of *T. melitensis*, it proves that there exists a specific diversity of *Tarzetia* in the Mediterranean area, adapted to the local vegetation.

T. quercus-ilicis differs from *T. melitensis*, probably associated with Mediterranean oak trees, by the shape of ascospores, ellipsoid vs. subfusoid, and their size, shorter in *T. quercus-ilicis*. It could also be confused with *T. cupularis* which can be found under *Quercus ilex*, but the latter is often short-stalked and its ascospores have a mean size that is shorter, with a Q ratio also shorter.

Tarzetia betulicola ad int. - Pl. 16 and fig. 5C.

Etymology: From Latin “*betula*”, meaning birch, and “*cola*” meaning “linked to”.

Apothecia 10–40 (55) mm diam., substipitate or shortly stipitate, deeply cupuliform, spreading at maturity; hymenium pallid beige; external surface pustulate, greyish to beige. **Margin** paler, eroded or denticulate. **Stipe** reaching 11 mm in length and 5.5 mm in diam.

Medullary excipulum ~700–800 µm thick, of *textura intricata*, with hyaline hyphae. **Ectal excipulum** ~150 µm thick, of *textura globulosa/subglobulosa*, with cells 13–44 µm diam., mixed with some clavate or subglobose elements. **Asci** 250–300 × 14–16 µm, cylindrical, long narrowed to the base, without crozier, operculate, inamyloid, 8-spored. **Paraphyses** filiform, (3) 4–6 µm diam., not enlarged at the top, straight, hyaline, simple or digitate, often with lateral bumps. **Ascospores** (17) 18–21 (22) × (9) 10–11.5 (12) µm, $X = 19.2 \times 10.5$ µm ($n=54$), $Q=(1.6)$ 1.7–2.1, $Q_m=1.8$, ellipsoid but rather elongated to subfusoid, hyaline, smooth, thick-walled, containing two large oil drops, with numerous small guttules, merged into one large guttule on rehydrated material.

Studied material: ANDORRA: Soldeu, 42.575475° N 1.657945° E, 1758 m a.s.l., on soil, under *Betula* sp. and *Pinus uncinata*, leg. M. Pélissier, 8 Jun. 2018, herb. M.P. 2018-133 (duplicata LY:NV 2018.06.49). ICELAND: Austurland, Hólarnir, Hallormsstaður, ~65.094° N 14.717° W, ~130 m a.s.l., on soil, in a birch forest, leg. Guðríður Gyða Eyjólfssdóttir, 2 Aug. 1993, herb. AMNH FA-14527 under *T. spurcata*.

Comments: Based on the ecology of the two studied collections, we think it is highly probable that this species grows in association with *Betula* trees. Macroscopically it looks like *T. catinus* or *T. ochracea*, but the ascospores are shorter and their shape is more elongated. Molecularly the LSU sequences of the examined collections fall within the same clade as *T. catinus*, along with *T. spurcata* suggesting that our current concept of *T. catinus* is a complex of species. Other genes may be necessary to resolve the taxonomy of this group. This is why we present this taxon ad interim.

Geopyxis albocinerea Velen. could be an older name for this species because its lectotype (PRM 150774) was collected under *Betula* in Czech Republic and synonymized with *T. gaillardiana* by SVRČEK (1979). Unfortunately, the Czech author indicated that the collection was fully immature and “partially destroyed by moulds”, so no comparison can be done with the studied material.



Plate 16 – *Tarzetia betulicola* ad int. Coll. MP 2018-133 (in situ). Photo M. Pélissier.

Excluded or doubtful species

Here is a list of species placed in *Tarzetia* that we exclude from this genus. The correct name is in bold.

Tarzetia ammophila (Sacc.) Theodor., *Badania Przyrodnicze Pomorskie, Tow. nauk., Torun:* 11 (1936).

≡ ***Peziza ammophila*** Sacc.

Tarzetia brasiliensis Rick, *Brotéria, sér. bot.,* 25 (2-3): 80 (1931).

The short diagnosis of RICK (1931) indicates “... *sporis* [...] *eguttulatis*.” This feature excludes it from the genus *Tarzetia*.

Tarzetia cinerascens Rehm, *Ann. mycol.,* 2 (4): 352 (1904).

The diagnosis of REHM (1904) indicates “*Asci*... *porus* J+”. This feature excludes it from the genus *Tarzetia*.

Tarzetia rapulum (Bull.) Rehm, *Rabenh. Kryptog.-Fl., Pilze – Ascom.,* 1 (3): 1021 (1894).

≡ ***Stromatinia rapulum*** (Bull.) Boud.

Tarzetia rapuloides Rehm, *Hedwigia,* 38 (Beibl.): (243) (1899).

The short size of ascospores, 8–10 × 5 µm, given by REHM (1899) seems doubtful for a *Tarzetia* and suggests a member of inoperculate discomycetes.

Tarzetia rosea (Rea) Dennis, *Brit. Ascom.,* 30 (1978).

≡ ***Rhodotarzetia rosea*** (Rea) Dissing & Sivertsen

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