

Velenovskya, a new genus in the family Pezizaceae to accommodate *Plicariella vacini*

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Abstract: Phylogenetic analyses based on partial sequences of the 28S rDNA and the rpb2 gene obtained from a collection of *Plicariella vacini* ($\equiv$ *Peziza vacini*) and homologous sequences from public databases suggest that this species represents an isolated lineage within the family *Pezizaceae*, quite distant from either *Plicariella* and *Peziza* s. str. In accordance with these phylogenetic results, a new name for this clade, the monotypic genus *Velenovskya*, is proposed around the new combination *Velenovskya vacini*. Phylogenetic and taxonomic relationships between *Velenovskya* and related genera are discussed, and an updated morphological description, including extensive macro- and micro-morphological images, is provided.

Keywords: Ascomycota, *Peziza*, *Plicariella*, phylogeny, taxonomy.

Riassunto: L'analisi filogenetica basata sui marcatori 28S rDNA e rpb2, condotta su una raccolta di *Plicariella vacini* ($\equiv$ *Peziza vacini*) e sequenze omologhe nei database pubblici, suggerisce che rappresenti un lineage isolato all'interno della famiglia *Pezizaceae*, piuttosto distante sia da *Plicariella* che da *Peziza* s.s. In base a questi risultati filogenetici, viene proposto il nuovo genere monotipico *Velenovskya* e la nuova combinazione *Velenovskya vacini* per ospitare l'unico rappresentante del genere ad oggi conosciuto. Vengono discusse le relazioni filogenetiche e tassonomiche tra *Velenovskya* e generi correlati e viene fornita una descrizione morfologica aggiornata, comprese dettagliate immagini macro e micromorfologiche.

Parole chiave : Ascomycota, *Peziza*, *Plicariella*, filogenia, tassonomia.

Introduction

As pointed out by VAN VOOREN (2020), the family *Pezizaceae* DuRoi. is mainly characterised by the amyloid reaction of the asci, the absence of carotenoid pigments in cells and the uninucleate ascospores [see also KORF (1972) and BERTHET (1964)]. Recently, a systematic overview of the *Pezizaceae* was given first by JAKLITSCH *et al.* (2016) who recognized 32 genera in this family, and then by EKANAYAKA *et al.* (2018) who reached 45 genera. Starting from NORMAN & EGGER (1999), many phylogenetic studies were carried out on this family, and especially on genus *Peziza* Dill. ex Fr. HANSEN *et al.* (2005) produced a multigene phylogeny based on LSU rDNA, rpb2 and β -tubulin sequences, and concluded that the genus *Peziza* is paraphyletic, consisting of many lineages. In the last few years the different lineages of *Peziza* s.l. were accommodated under different generic names available or given new ones (e.g. VAN VOOREN *et al.*, 2015; AGNELLO *et al.*, 2018; YUAN *et al.*, 2020; VAN VOOREN, 2020).

In this new context, the most suitable taxonomic treatment for many species lacking sequence data still needs to be ascertained. One such species is *Plicariella vacini* Velen., originally described by VELENOVSKÝ (1939) upon samples collected on burnt ground in the Czech Republic. This species was later transferred to *Peziza* by SVRČEK (1977) as *Peziza vacini* (Velen.) Svrček after the re-examination of the holotype, a decision confirmed later by MORAVEC & SPOONER (1988), who also reviewed the holotype.

The aim of the present study is to infer the most suitable taxonomic treatment for *Plicariella vacini* using newly obtained DNA sequences from a collection of this species.

Material and methods

Morphological study. — Fruitbodies were photographed *in situ* with a Sony alpha 7rIII digital camera equipped with a Sony SEL90M28G macro 90 mm lens. The description and study of macro- and micro-morphological characters was carried out by observing both fresh and dried material. Ammonia Congo red and anionic Congo red were used to stain and highlight the microscopic elements. Melzer's reagent was used to determine the amyloid reaction of the asci prior to staining, exsiccata were hydrated in distilled water. The microscopic observations were done with two Leica DM 750 optical microscopes, with Leica HI PLAN or Leica DM 2000 N PLAN objectives providing 5 $\times$, 10 $\times$, 20 $\times$, 40 $\times$, 63 $\times$ and 100 $\times$ (immersion), and equipped with D.I.C. Ascospores measurements ($n = 30$)

and all other measurements of micro-elements were performed in water with the Piximètre program. The dried collection is housed in the Turku University Herbarium (TUR-A, Finland).

Phylogenetic studies. — Total DNA was extracted from dry specimens employing a modified protocol based on MURRAY & THOMPSON (1980). PCR reactions (MULLIS & FALOONA, 1987) included 35 cycles with an annealing temperature of 54°C. The primers ITS1F and ITS4 (WHITE *et al.*, 1990; GARDES & BRUNS, 1993) were employed to amplify the ITS rDNA region, LR0R and LR5 (VILGALYS & HESTER, 1990; CUBETA *et al.*, 1991) were used for the 28S rDNA region, and bRPB2-6F2 (reverse of bRPB2-6R2), and bRPB2-7R2 for the RNA polymerase II second largest subunit (rpb2) gene (MATHENY *et al.*, 2007). PCR products were checked in 1% agarose gels, and amplicons were sequenced with one or both PCR primers. Sequences were corrected to remove reading errors in chromatograms.

A combined 28S rDNA (LSU) – RPB2 – β -tubulin (TUB2) alignment was built using sequences from O'DONNELL *et al.* (1997), HANSEN *et al.* (2001, 2005), CABERO *et al.* (2016), VAN VOOREN *et al.* (2018), VAN VOOREN (2020) and PAZ *et al.* (2022) retrieved from the International Nucleotide Sequence Database Collaboration (INSDC) public database (COCHRANE *et al.*, 2011), and new sequences obtained during this study. Sequences were first aligned in MEGA 5.0 (TAMURA *et al.*, 2011) software with its Clustal W application and then corrected manually. The final alignment included 131 sequences of 28S rDNA, 82 sequences of RPB2 gene, and 61 sequences of β -tubulin. The aligned loci were loaded in MrBayes 3.2.6 (RONQUIST *et al.*, 2012), where a Bayesian analysis was performed (data partitioned into LSU, RPB2 exons and β -tubulin exons, two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after 2.18 M generations, standard deviation having fallen below 0.01. Finally a full search for the best-scoring maximum likelihood tree was performed in RaxML 8.2.12 (STAMATAKIS, 2014) using the standard search algorithm (data partitioned, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Phylogenetic results

The combined analysis of LSU, RPB2 and TUB2 sequences of the main clades of the family *Pezizaceae* produced a topology (Fig. 1) similar to that obtained by previous researchers (VAN VOOREN, 2020; PFISTER *et al.*, 2022). The sample of *Plicariella vacini* showed a signifi-

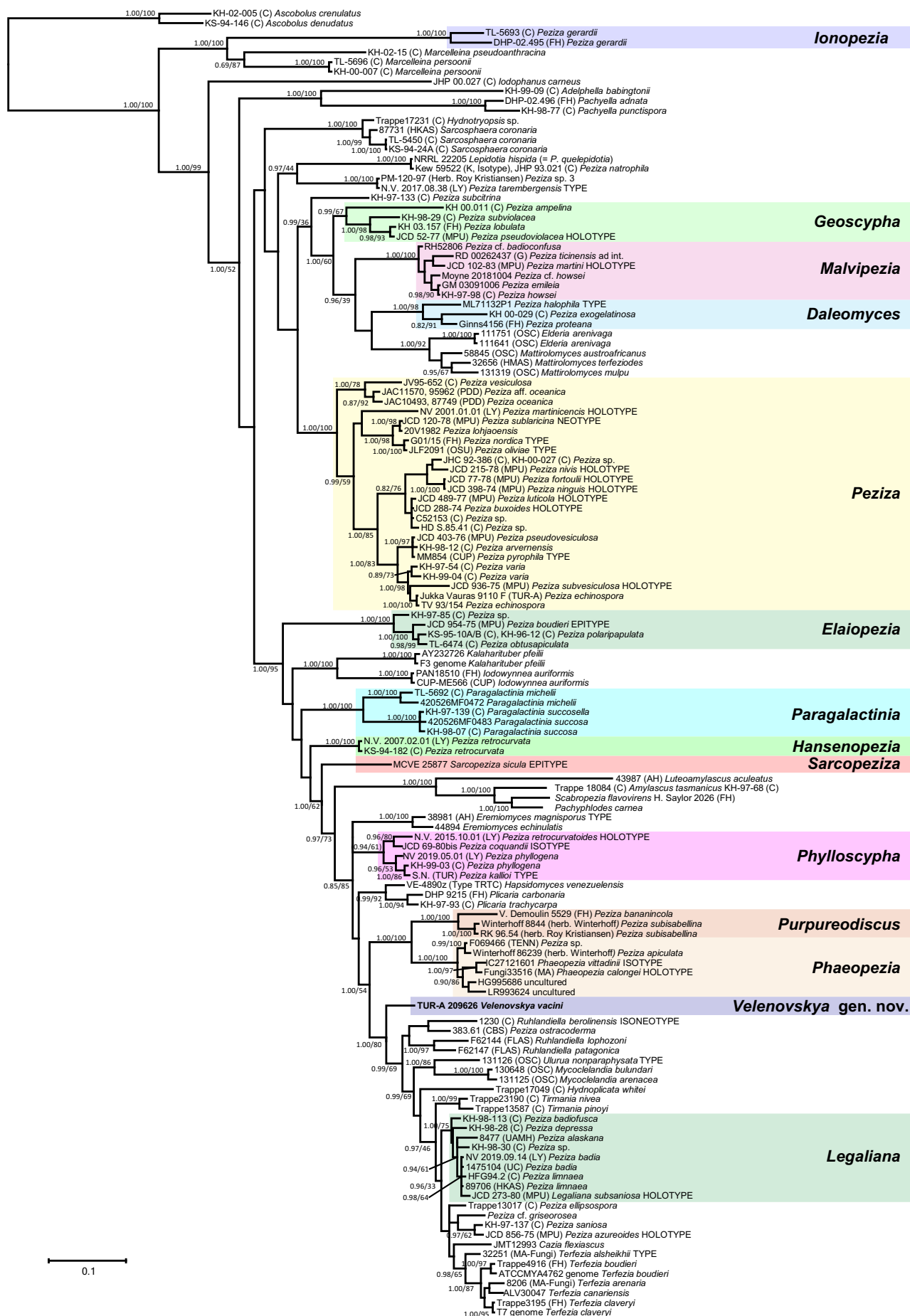


Fig. 1 – A 50% majority rule 28S rDNA - RPB2 - TUB2 consensus phylogram of the family *Pezizaceae* (with selected species of *Ascobolus* Pers. ex J.F. Gmel., *Ascobolaceae* as outgroup) obtained using MrBayes from 16350 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or $\geq 70\%$ maximum likelihood bootstrap proportions (right). The sample sequenced in this study is highlighted in bold.

cant similarity (1.00 PP, 80 BP) to the clade containing the genus *Legaliana* Van Vooren and multiple sequestrate genera, such as *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul. and *Tirmania* Chatin, as well as *Cazia* Trappe, *Hydnoplicata* Gilkey, *Mycoclelandia* Trappe & G.W. Beaton, *Ruhlandiella* Henn. and *Ulurua* Trappe, Claridge & Kovács. To a lesser extent, these clades are also close to the genera *Phaeopezia* (Vido) Vido and *Purpleodiscus* (G. Hirsch) Van Vooren, the first one containing both species with cup-shaped and sequestrate ascomata (Paz *et al.*, 2022). The genetic distance observed between *Plicariella vacini* and the closest clades can be considered more or less similar to that displayed between different lineages of other genera inside the family *Pezizaceae* (i.e. *Peziza* s. str.). However, the numerous morphological differences between *Plicariella vacini* and the lineages related to it, and the important tradition of the names applied to some of these genera discourage the reorganization of all these clades into a single genus. A paraphyletic genus *Peziza* is also rejected here to avoid confusion, as names for most major clades were already proposed by VAN VOOREN (2020). Since genus *Plicariella* is currently considered related to or even nested inside genus *Pachyphlodes* Zobel (NORMAN & EGGER, 1999; HANSEN *et al.*, 2005; LÆSSØE & HANSEN, 2007; HEALY *et al.*, 2009, 2017; LI *et al.*, 2019; LIU *et al.*, 2020), the clade of *Plicariella vacini* is given below a new name, and this species is combined into the new genus *Velenovskya*.

Taxonomy

Velenovskya Albanese, Boragine, M. Carbone & P. Alvarado, *gen. nov.* – MB 844786

Diagnosis: Apothecia discoid or irregular in outline, flattened to

more or less convex, smooth to cerebriform; ascospores initially hyaline then yellow-brownish at maturity, ornamented; asci operculate, lightly amyloid in almost their entire length, much deeper at the top, 8-spored, with croziers; paraphyses cylindrical, septate, bifurcated, rarely trifurcated, with apex of various shape immersed in a light-brownish gelatinous content; subhymenium thin, of a dense *textura intricata*; medullary excipulum of *textura intricata* of cylindrical, subhyaline hyphae mixed with pyriform to rounded cells in the outer part; ectal excipulum of *textura angularis*. Besides its unique genetic profile, it differs from *Plicariella* in having ellipsoid ascospores, and from *Peziza* s. str. in having heavily ornamented light-brownish ascospores at maturity.

Etymology: Dedicated to Josef Velenovský (22 April 1858 – 7 May 1949), who first described the type species, *Plicariella vacini*.

Type: *Velenovskya vacini* (Velen.) Albanese, Boragine, M. Carbone & P. Alvarado, *comb. nov.* – MB 844787

Basionym: *Plicariella vacini* Velen., *Novitates Mycologicae*: 198 (1939).

Homotypic synonym: *Peziza vacini* (Velen.) Svrček [as ‘*vacinii*’], *Česká Mykol.*, 31 (2): 70 (1977).

Typification: Holotype: Czechia, South Moravia, near Žarošice, on burnt soil, VIII.1939, leg. V. Vacek (as ‘*Vacinus*’), herb. PRM 151840. Reviewed by SVRČEK (1979) and MORAVEC & SPOONER (1988).

Original description: *Ap. sparsa, ca 1 cm diam., cito patellaria, carnosa, sessilia, olivacea, nuda. As. 250×15, jodo coerulei, par. simpl. filiformes, apice 3-5, omnes cohaerentes. S. 15-18, sub-globosae, grosse*



Plate 1 – *Velenovskya vacini*. TUR-A 209626. A: samples *in situ*; B: detail of flesh; C: hymenial surface; D: external surface. Photos: A. Albanese.

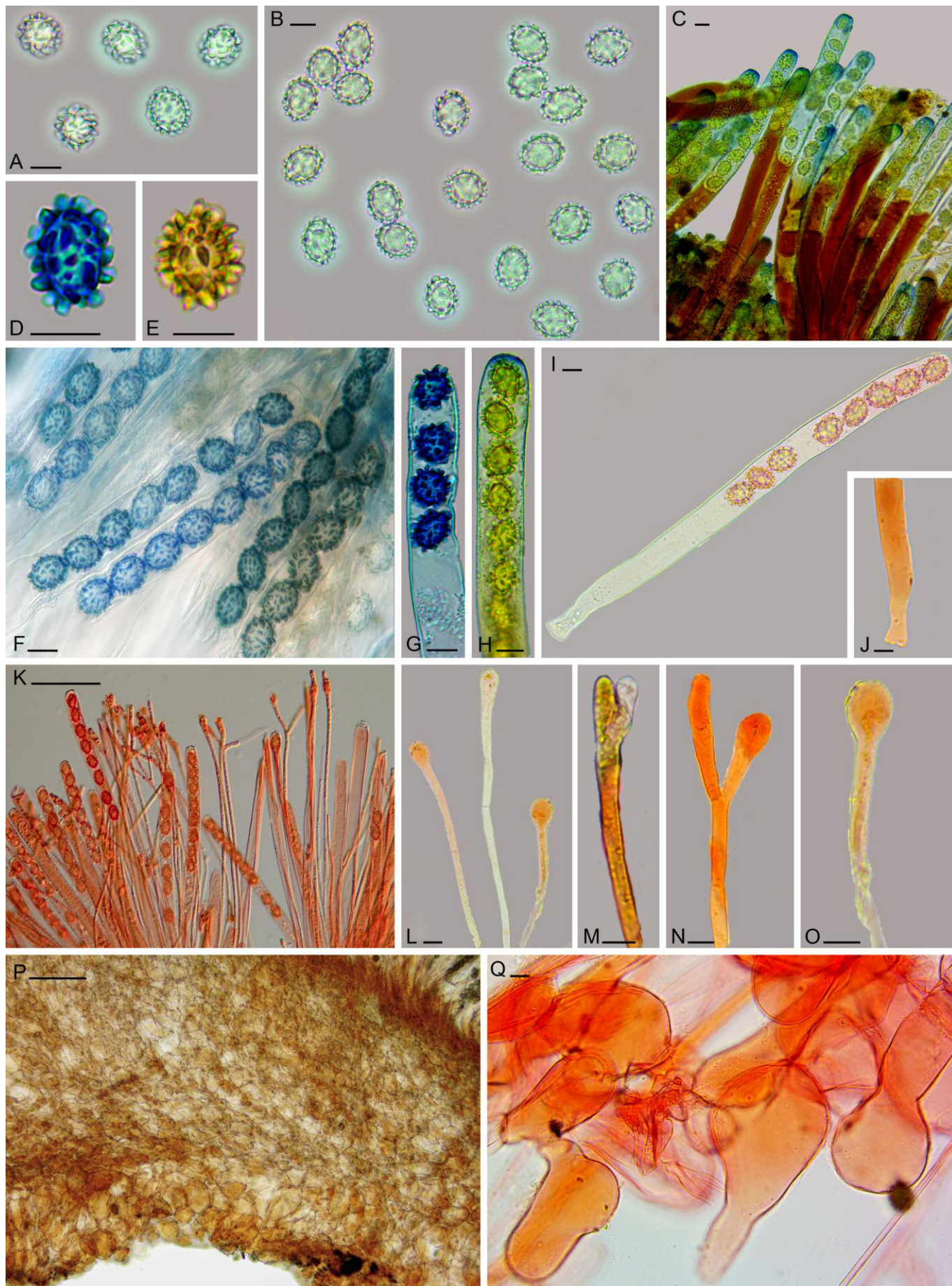


Plate 2 – *Velenovskya vacini*. TUR-A 209626. Microscopic features. A–B: ascospores in water; C: asci in Melzer's; D: ascospore in cotton blue; E: brownish ascospore in water; F–G: ascospores in cotton blue; H: upper part of ascus in Melzer's; I: ascus in Congo red; J: ascus base; K–O: paraphyses in Congo red; P: section of an apothecium in water; Q: pyriform elements of the outer medullary excipulum in Congo red. All bars = 10 μ m, excluding P and K = 100 μ m. Photos: A. Albanese & M. Boragine.

verrucosae, fusce, 1–2 guttulate. In carbonario pr. Zarosice Moraviae leg. amicus Vacinus aug. 1939.

Nomenclatural note: The right epithet of this species is *vacini* and not *vacinii* as Velenovský dedicated it to his friend Vacek, latinised in *Vacinus* and so the right spelling is *vacini* as rightly reported in the protologue.

Macroscopical features: **Apothecia** gregarious, discoid or irregular in outline, up to 22 mm in diam., sessile, flattened to more or less convex. **Hymenium** smooth to cerebriform, light brown to brown-hazel or dark brown with violet tinges. **External surface** subconcolour to the hymenium, with greenish tinges. **Context** with a waxy-gelatinous consistency, up to 3 mm thick, beige-gray with purple hues; smell and taste not tested.

Microscopic features: **Ascospores** initially hyaline then yellow-brownish, largely ellipsoidal, uniguttulate but also biguttulate, $(12.2\text{--}12.6\text{--}14.5\text{--}14.8) \times (8.6\text{--}9.7\text{--}10.5\text{--}13) \mu\text{m}$ (without ornaments), $Q = (1\text{--})1.2\text{--}1.4\text{--}(1.6)$ [$N = 30$], $av. = 13.5 \times 10.6 \mu\text{m}$; $Q_m = 1.3$, ornamented by coarse and thick wing-like obtuse to pyramidal warts, $1.5\text{--}2.7 \mu\text{m}$ high, which sometimes coalesce to create a pseudoreticulum. **Asci** cylindrical, $233\text{--}252 \times 14\text{--}16.8 \mu\text{m}$, operculate, slightly amyloid in almost their entire length, much deeper at the top [WT type according to VAN VOOREN (2020); “i” type according to HANSEN *et al.* (2001)], 8-spored, with croziers. **Paraphyses** cylindrical, septate, bifurcated at the top, rarely trifurcated, with variously shaped apices ranging from clavate to lanceolate or very capitate, $6.1\text{--}13.5 \mu\text{m}$ in diam.; immersed in a light brownish gelatinous matrix. **Subhymenium** thin, made of a dense *textura intricata* of septate hyphae. **Medullary excipulum** bilayered: 1) in the inner part, of *textura intricata* made of subhyaline, cylindrical hyphae, arranged in parallel to the hymenial surface, $14.6\text{--}25 \mu\text{m}$ in diam., with walls up to $0.5 \mu\text{m}$ thick; 2) the outer part resembling a *textura globulosa*, formed by septate and intertwined cylindrical hyphae, $7.7\text{--}12.6 \mu\text{m}$ in diam., and pyriform to rounded cells, $33.3\text{--}56.5 \mu\text{m}$ in diam., with walls up to $0.7 \mu\text{m}$ thick. **Ectal excipulum** of *textura angularis*, composed by elements of $128\text{--}23.5 \mu\text{m}$ in diam.

Habitat and phenology: Our specimens were collected in winter (January) during a field trip on the “Domitio” shoreline (Campania, Italy). Temperature was 13°C (85% of humidity) and the ground was very moist due to a lot of rainfall in the previous days; soil is mostly sandy and siliceous (acidic). Ascومات grew on abundant charred remains of *Pinus halepensis*, on the edge of a 50 m^2 area completely flooded by the rain.

Distribution: The species seems to be unfrequent and to date it has been recorded in Czech Republic (VELENOVSKÝ, 1939; MORAVEC & SPOONER, 1988), Switzerland (BLANK & DOUGOUD, 1991; ROFFLER, 2001), Great Britain (MORAVEC & SPOONER, 1988), Norway (KRISTIANSEN, 1982), Latvia (VIMBA & RAITVIIR, 2006), Germany (HOHMEYER *et al.*, 1989; BENKERT, 1991) and France (MOYNE & MOINGEON, 2017; HAIRAUD, 2021). According to MOYNE & MOINGEON (2017) it is present also in Spain and Austria. We are not aware of any official record from Italy, so our collection could be regarded as the first for this country.

Studied and sequenced collection: ITALY. Campania, Cellole (CE), Loc. Sora Maria, $41^\circ13'23.5''\text{N}$, $13^\circ45'59.8''\text{E}$, alt. 10 m a.s.l., on burnt ground close to *Pinus halepensis*, *Cistus salvifolius*, *Smilax aspera*, *Olea europea* and *Juniperus communis*, 05.I.2022, A. Albanese (TUR-A 209626), Genbank codes: ITS = ON775471; LSU = ON775566; rpb2 = ON758342.

Discussion

Velenovskya vacini was described by VELENOVSKÝ (1939) as *Plicariella vacini*. The genus *Plicariella* (Sacc.) Rehm was typified with *Peziza*

radula Berk. & Broome by ECKBLAD (1968), who considered it a later synonym of *Plicaria* Fuckel. However, SPOONER (2001) reviewed the holotype of *Peziza radula* and concluded that *Plicariella* is different from *Plicaria*, and should be regarded an earlier synonym of *Scabropezia* Dissing & Pfister. In addition, *Plicariella* (= *Scabropezia*) was considered either a sister lineage of *Pachyphlodes* Zobel (NORMAN & EGGER, 1999; HANSEN *et al.*, 2005), or embedded within it (LÆSSØE & HANSEN, 2007; HEALY *et al.*, 2009, 2017; LI *et al.*, 2019; LIU *et al.*, 2020). Whatever the actual relationship between these genera is, the lineage of *Plicariella vacini* is obviously unrelated to them (Fig. 1) and so the new name *Velenovskya* is here proposed.

Velenovskya vacini [as *Peziza “vacinii”*] has been treated by several authors in the past (HOHMEYER, 1986; MORAVEC & SPOONER, 1988; HOHMEYER *et al.*, 1989; BENKERT, 1991; BLANK & DOUGOUD, 1991; DISSING, 2000; ROFFLER, 2001; VIMBA & RAITVIIR, 2006; MOYNE & MOINGEON, 2017; HAIRAUD, 2021). This uncommon species cannot be confused with any other anthracophilous taxon because of its striking and unique macro- and micro-morphological features (see above). Our collection fits very well the protologue (spores shape apart) and later studies of the type material conducted by SVRČEK (1979) and MORAVEC & SPOONER (1988). VELENOVSKÝ (1939) described the ascospores as “15–18, sub-globosae”, but SVRČEK (*op. cit.*) and MORAVEC & SPOONER (*op. cit.*) suggested that this statement is wrong or at least misleading. In addition, as already pointed out by MORAVEC & SPOONER (*op. cit.*), the spores drawn by SVRČEK (*op. cit.*) are also misleading because they do not display accurately the height of the warts, typical of this species.

As reported above in the phylogenetic results, the genus *Velenovskya* shows a significant similarity to the clade containing the genus *Legaliana* and several sequestrate genera. *Legaliana* has been described to accommodate *Peziza badia* Pers. and related species. It is mainly characterised by dark coloured epigeous ascومات, producing variously ornamented ascospores, asci with a WT type amyloid reaction, and an excipulum without a gelled outer layer (VAN VOOREN, 2020; VAN VOOREN *et al.*, 2021). The genus *Chromelosporiopsis* Hennebert (HENNEBERT, 2020; PFISTER *et al.*, 2022), described from anamorphic samples, is apparently closely related to *Legaliana*, and its actual status should be further explored (in case of synonymy, *Legaliana* should have priority because of its earlier publication date). Regarding the ancient genus *Chromelosporium* Corda, the phylogenetic placement of the type species, *C. ochraceum* Corda, still needs to be clarified, but PFISTER *et al.* (2022) report that the species *C. macrospermum* Hennebert is related to *Peziza ostracoderma* Korf. In the phylogeny obtained in the present work, the latter has no clear affinities, but it is maybe related to genus *Ruhlandiella*, and in any case, it is significantly different from *Velenovskya*.

Regarding the sequestrate genera related to *Legaliana*, *Ruhlandiella* is a genus of exothecial, ectomycorrhizal fungi, producing spherical ornamented spores and dextrinoid asci, inamyloid to weakly amyloid in some species (KRAISITUDOMSOOK *et al.*, 2019). *Hydnoplicata* is another sequestrate genus containing by now a single Australian species *Hydnoplicata convoluta* (McAlpine) Trappe & Claridge (= *Hydnoplicata whitei* Gilkey); it is distinguished mainly by the non-operculate, diffusely amyloid asci and slightly ornamented ascospores (TRAPPE & CLARIDGE, 2006). *Mycoclelandia* contains two endemic Australian desert truffles, *M. arenacea* (Trappe) Trappe & G.W. Beaton and *M. bulundari* (G.W. Beaton) Trappe & G.W. Beaton, which are characterised by diffusely amyloid asci and smooth ascospores (TRAPPE *et al.*, 2010; CLARIDGE *et al.*, 2014). *Ulurua* is a monotypic genus erected for the Australian desert truffle *Ulurua nonparaphysata* Trappe & Claridge, characterised by the lack of paraphyses in the hymenium, in combination with the diffusely amyloid asci and ornamented ascospores (TRAPPE *et al.*, 2010). According to ALSHEIKH & TRAPPE (1983), the desert truffle genus *Tirmania* is characterised by the amyloid reaction of the asci combined with a double ascospore wall, the outer part being smooth and the inner part with a reticulate-roughened wall, while according to DIEZ *et al.*

(2002) the closely related “desert truffle” genus *Terfezia* is distinguished by the non-amyloid asci and the variously ornamented ascospores. The genus *Cazia* comprises two hypogeous north-western American species *Cazia flexiascus* Trappe and *C. quericola* Fogel & States, and it is mainly characterised by inamyloid asci (TRAPPE, 1989; FOGEL & STATES, 2002, as “*Cazia quericola*”).

Other taxa more distantly related to *Velenovskya* include the genus *Phaeopezia*, which comprises both cupulate and sequestrate species (PAZ *et al.*, 2022). It is mainly characterised by eguttulate ascospores, and asci with a WT type amyloid reaction in the apothecial species and inamyloid in the sequestrate ones (VAN VOOREN, 2020; VAN VOOREN *et al.*, 2021; PAZ *et al.*, 2022).

Finally, the genus *Purpureodiscus* comprises species with a habit resembling *Pachyella*, asci with wall diffusely bluing in an iodine solution (W type), ascospores smooth or ornamented by isolated warts, in some species germinating to directly produce conidia (VAN VOOREN & VALADE, 2006; VAN VOOREN, 2020).

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Authors' contributions

Matteo Carbone is responsible for the writing of the first draft, which was subsequently updated by all authors. Morphological analyses were performed by Antonio Albanese and Marcello Boragine. Molecular analyses and all the phylogenetic chapters and notes were performed by Pablo Alvarado (ALVALAB). Plates were assembled by M. Carbone using the pictures taken by A. Albanese and M. Boragine. All authors read and approved the final manuscript.

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