


Sarcopeziza (Pezizaceae, Ascomycota), a new monotypic genus for Inzenga's old taxon *Peziza sicula*

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Abstract: Phylogenetic inferences in recent years have shown that the genus *Peziza*, in its traditional circumscription, is polyphyletic. Multigene phylogenetic analyses based on the ITS and 28S rDNA, as well as *rpb2* and *tef1* loci carried out on the epitype collection of *Peziza sicula*, suggest that it represents an isolated lineage within *Pezizaceae*, quite distant from *Peziza* s. str. To reflect phylogenetic results, the new monotypic genus *Sarcopeziza* is proposed, and the new combination *Sarcopeziza sicula* is provided to accommodate the sole representative of the genus so far. Phylogenetic and taxonomic relationships between *Sarcopeziza* and related genera are discussed, and an updated morphological description, including extensive macro- and micromorphological images, is provided.

Keywords: *Pezizales*, molecular phylogeny, *Sarcosphaera sicula*, systematics, taxonomy.

Σύνοψη: Φυλογενετικές μελέτες τα τελευταία χρόνια έχουν δείξει ότι, με βάση τον παραδοσιακό του ορισμό, το γένος *Peziza* είναι πολυφυλετικό. Μοριακές αναλύσεις του γονιδίου 28S rDNA, καθώς και των γονιδίων *rpb2* και *tef1* rDNA που έγιναν στον επίτυπο του είδους *Peziza sicula*, έδειξαν ότι βρίσκεται σε απομονωμένη φυλογενετική θέση στην οικογένεια *Pezizaceae*, αρκετά μακριά από το γένος *Peziza* s. str. Προς εναρμονισμό με τα μοριακά δεδομένα, το καινούργιο μονοτυπικό γένος *Sarcopeziza* gen. nov. περιγράφεται πιο κάτω, και ο νέος συνδυασμός *Sarcopeziza sicula* comb. nov. προτείνεται για τον μοναδικό εκπρόσωπο του γένους μέχρι τώρα. Οι φυλογενετικές και ταξινομικές σχέσεις του καινούργιου γένους με παραπλήσια γένη αναλύονται, ενώ αναθεωρημένες μορφολογικές περιγραφές, καθώς και εκτεταμένη εικονογραφία, επισυνάπτονται.

Introduction

The order *Pezizales* J. Schröt. (ENGLER & PRANTL, 1894: 173), includes about 200 morphologically diverse genera producing epigeous, hypogeous, or semi-hypogeous ascomata with operculate asci (LUTZONI *et al.*, 2004; HANSEN *et al.*, 2005; HANSEN & PFISTER, 2006). Pezizoid species are widespread in temperate and alpine regions (HANSEN *et al.*, 2005; HANSEN & PFISTER, 2006), but several Mediterranean and tropical species have also been described (PFISTER, 1991; VIZZINI *et al.*, 2016; CROUS *et al.*, 2017). Although pezizoid fungi were traditionally considered to be saprophytes, recent studies revealed that many species form ectomycorrhizal or endophytic associations with vascular plants (WARCUP, 1990; TEDERSOO *et al.*, 2006, 2010, 2013; SMITH *et al.*, 2007; LOIZIDES *et al.*, 2016). As a result, pezizoid fungi can be found in diverse habitats and substrates, from calcareous and acidic soils, to logs, plant debris, charcoal, sand dunes, salt marshes, dung, straw, or masonry, while species growing in urine or submerged in water have also been described (HANSEN & PFISTER, 2006; MEDARDI, 2006; FRANK, 2013; VAN VOOREN *et al.*, 2017).

The species in the family *Pezizaceae* Dill. ex Fr. (DUMORTIER, 1829) are characterized by ascospores which are usually ornamented with cyanophilic ridges or warts, and have amyloid asci, although this feature has been lost in some lineages (HANSEN *et al.*, 2001). In the late 20th and early 21st centuries, *Pezizaceae* fungi have attracted the interest of several phylogenetic studies (NORMAN & EGGER, 1996, 1999; LANDVIK *et al.*, 1997; HANSEN *et al.*, 2002; HANSEN & PFISTER, 2006). Phylogenetic reconstructions revealed at least fourteen clades within *Pezizaceae*, which could be considered independent genera (HANSEN *et al.*, 2005). Spore ornamentation and guttulation, excipulum structure and pigmentation, as well as the intensity and distribution of the amyloid reaction of the ascus wall, were shown to be important synapomorphic features that can be used to discriminate between these clades (HANSEN *et al.*, 2001, 2002). These studies also showed that the largest genus in the family, *Peziza* Dill. ex Fr., is not a monophyletic lineage, as species ascribed to this genus occur in at least eight of the putative genera identified, often related with hypogeous or semi-hypogeous angiocarpic species (O'DONNELL *et al.*, 1997; PERCUDANI *et al.*, 1999; HANSEN & PFISTER, 2006). Nevertheless, no comprehensive systematic revision of *Pezizaceae* has been proposed to date and taxonomic arrangements, particularly within the type genus *Peziza*, remain problematic. PFISTER *et al.* (2016) recently advocated to use the generic names *Adelphella* Pfister, Matočec & I. Kušan, *Galactinia* (Cooke) Boud., and *Lepidotia* Boud., for several

taxa previously placed in *Peziza* s. lato, paving the way for a more focused taxonomic arrangement in *Pezizaceae* more accurately reflecting current phylogenies.

In the present study, we sought to clarify the phylogenetic and taxonomic position of *Peziza sicula* Inzenga (1869). This outstanding taxon shares many morphological similarities with *Sarcosphaera coronaria* (Jacq.) J. Schröt., because of its initially hypogeous growth and hollow angiocarpic ascomata later tearing into star-shaped lobes (lacinias) and was in the past synonymized with the latter species by SACCARDO (1889). This decision was not shared by PATOUIL-LARD (1904), however, who studied a Tunisian collection of this species, nor by BOUDIER (1907) who considered it an independent species in the genus *Sarcosphaera* Auersw. (1869). *Peziza sicula* was recently epitypified from contemporary samples collected in Salento (Apulia, Italy) by AGNELLO *et al.* (2013, 2015), but until now phylogenetic data for this species was lacking. In the present work, new collections from Cyprus and mainland Greece are studied. Morphology, ecology, and multigene phylogeny are used to determine the most suitable generic placement of this species and update its taxonomy.

Material and methods

Morphological studies. — Specimens were photographed in situ and detailed notes on macroscopic features were taken from fresh material. Microscopic characters were studied from fresh and dried specimens. Two optical microscopes were used: an Optika trinocular with plan-achromatic objectives, and a Leica BM E binocular, at 40×, 100×, 400×, 1000× magnifications. The following reagents were used as mounts: Melzer's reagent for amyloidity; cotton blue for spore ornamentation; Congo red for the highlighting of hyaline micro-structures; water mounts to observe the pigmentation and measure of the spore size. Ascospores were measured in water, from naturally ejected spores. At least 30 spores from each spore print were measured and the Q (minimum and maximum length/width ratio), and Qm (mean length/width ratio) were calculated.

DNA extraction, amplification and sequencing. — Total DNA was extracted from dry specimens employing a modified protocol based on MURRAY & THOMPSON (1980). PCR amplification was performed with the primers ITS1F and ITS4 (WHITE *et al.*, 1990; GARDES & BRUNS, 1993) for ITS region, while LR0R and LR5 (VILGALYS & HESTER, 1990; CUBETA *et al.*, 1991) were used to amplify the 28S rDNA region,

EF1-983F and EF1-1567R (REHNER & BUCKLEY, 2005) for the translation elongation factor 1a (*tef1*) gene, and bRPB2-6F and bRPB2-7R2 for the RNA polymerase II second largest subunit (*rpb2*) gene (LIU *et al.*, 1999; MATHENY *et al.*, 2007). Chromatograms were checked searching for putative reading errors, and these were corrected.

Phylogenetic analysis. — BLAST (ALTSCHUL *et al.*, 1997) was used to select the most closely related 28S rDNA, RNA polymerase II second largest subunit (*rpb2*), and β -tubulin (*btub*) sequences from INSD public databases. Sequences came mainly from HANSEN *et al.* (2005) and KOVACS *et al.* (2011). Sequences first were aligned in MEGA 5.0 (TAMURA *et al.* 2011) software with its Clustal W application and then corrected manually. The final alignment included 345/939 (28S rDNA), 836/1704 (*rpb2*) and 262/772 (*btub*) variable sites. The aligned loci were loaded in PAUP* 4.0b10 (SWOFFORD, 2001) and a maximum parsimony phylogenetic tree reconstruction was performed (2000 bootstrap replicates, TBR swapping algorithm, 50 sequence additions per replicate, MULTREES not in effect). Aligned loci also were subjected to MrModeltest 2.3 (NYLANDER, 2004) in PAUP* 4.0b10. Model GTR+I+G was selected and implemented in MrBayes 3.1 (RONQUIST & HUELSENBECK, 2003), where a Bayesian analysis was performed (data partitioned, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 0.59M generations, standard deviation having fell below 0.01. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML (STAMATAKIS, 2006) using the standard search algorithm (data partitioned, GTRMIX model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Terminology, nomenclature and fungarium material. — Terminology of different angiocarpic forms follows HANSEN *et al.* (2001), applying the term “ptychothecia” to hollow or chambered ascomata with a discernible hymenium of asci and paraphyses typically lining

the hollow chambers; the term “stereothechia” to solid ascomata without a regularly arranged hymenium but with asci randomly distributed throughout the gleba tissue; and the term “exothecia” to solid ascomata on which the hymenium covers the external surface. Classification of the different types of ascus amyloidity follows the same authors and is further discussed in the next chapter. Unless otherwise noted, nomenclature follows Index Fungorum (www.indexfungorum.org). Exsiccata are housed at Museo di Storia Naturale di Venezia (MCVE) and in the private collections of the authors.

Results

Results based on 28S rDNA and *rpb2* sequences obtained from *Peziza sicula* suggest that this species represents a genetic lineage not directly related with that of the type species of *Peziza*, *P. vesiculosa* Bull. : Fr. (HANSEN *et al.*, 2005; PFISTER *et al.*, 2016). This core lineage or *Peziza s. str.* is subsignificantly related with other species of *Peziza*, and probably also to some hypogeous genera such as *Mattiolomyces* and *Elderia*, as suggested by preliminary ML analyses based on 28S rDNA sequences available in public databases (data not shown) and other researchers (LÆSSØE & HANSEN, 2007; HEALY *et al.*, 2013). However, *P. sicula* is related to a different clade, formed by a diverse group of genera including *Galactinia*, *Iodowynnea*, the clade of *Pachyphloides*, *Scabropezia*, *Amylascus* and *Luteoamylascus*, the clade of *Plicaria* and *Hapsidomyces*, *Ruhlandiella*, the clade of *Terfezia*, *Tirmania* and *Hydnoplicata*, and probably also the hypogeous genera *Eremiomyces*, *Cazia*, *Calongea*, *Ulurua*, *Mycoclelandia*, and *Kalaharituber* (LÆSSØE & HANSEN, 2007; HEALY *et al.*, 2013).

Genetic results suggest that *P. sicula* could represent an independent lineage of *Pezizaceae* with a partially sequestrate or angiocarpic habit that evolved from a *Peziza*-like ancestor. Merging *P. sicula* and other ancient sequestrate genera with their pezizoid

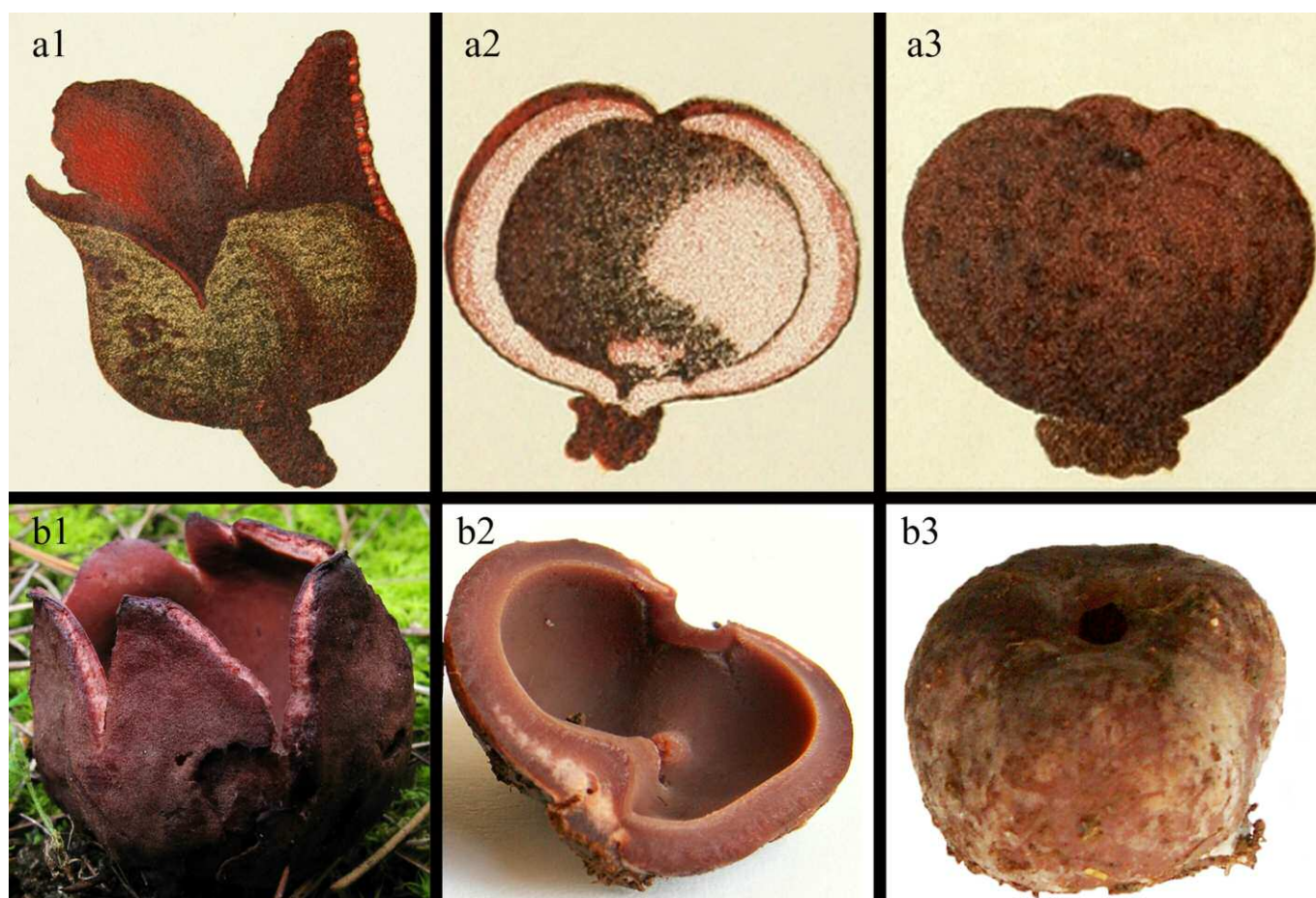


Fig.1 – Comparison between new collections and the ancient plate of Inzenga (a1, a2, a3); b1: Photo in habitat from Greece (I. Paradis); b2, b3: Study photos of young samples from Italy (C. Agnello).

relatives in a single genus does not seem practical, and so the best option seems to accommodate all major lineages into distinct genera, following PFISTER *et al.* (2016). Therefore, the new genus *Sarcopeziza* is proposed to accommodate this species.

Taxonomy

Sarcopeziza Loizides, Agnello & P. Alvarado, *gen. nov.* – MB 827573 – Figs. 1–5

Diagnosis: Ascomata hypogeous or semi-hypogeous, medium- to large-sized, sarcosphaeroid in aspect and distinctly or indistinctly stipitate, at first angiocarpic, hollow, subglobose to pyriform, with a small apical orifice, gradually tearing concentrically to form erect or arched lacinias; hymenial surface smooth, purplish-violet; asci with fully functioning opercula, pleurorhynchous, diffusely amyloid over the entire length, more intensely at the apex; paraphyses multiseptate, cylindrical or sometimes moniliform; ascospores monoseriate, ellipsoid, smooth or finely low warty, hyaline or subhyaline and mono- or multiguttulate; in xeric scrub, dry grasslands and thermomediterranean matorral.

Etymology: From Greek, σάρξ, *sarx*, meaning flesh, and πεζίς, *pezis* for the similarity with species of the genus *Peziza*.

Type species: *Sarcopeziza sicula* (Inzenga) Agnello, Loizides & P. Alvarado, *comb. nov.* – MB 827574

Basionym: *Peziza sicula* Inzenga, *Funghi Siciliani, Centuria II*: 39 (1869).

Homotypic synonym: *Sarcosphaera sicula* (Inzenga) Pat., *Bulletin Société d'histoire naturelle d'Autun*, 17: 154 (1904).

Original description

Peziza majuscula rubro-fusca, cupula globosa, ore angusto contracto, dein expansa campanulata, inaequaliter lacero-multifida stipiteque brevi subnullo.

Descrizione. La *Peziza sicula* è di forma molto grossa e sviluppata, con la Cupula, nel suo primo sviluppo, di color rosso pavonazzo all'esterno, nell'interno di color carneo, sferica, spesso longitudinalmente solcata, colla bocca o apertura circolare molto ristretta ed infossata, quasi sessile; nello stato adulto presentasi stipitata, a Stipite corto, solido, ricurvo, fessa longitudinalmente dall'alto al basso in diverse sezioni, aperte, ripiegate indietro, lacero-dentate ovvero lacero seghettate nel loro contorno, e che prende la forma campanulata-multifida. Il suo Desco o la sua superficie interna da principio color carneo trasmutasi allora in color rosso-pavonazzo: come l'esterna sua superficie da principio color rosso-pavonazzo diviene di colore ocreo, chiazzato irregolarmente di macchie sanguigne. Nell'uno e nell'altro stadio vegetativo, presentasi il fungo di consistenza ceracea, nell'esterna sua superficie sempre disuguale, ovvero grinzoso-lacunosa. Asci costantemente ottospore ripiene di Sporule ovali, semplici, simmetricamente inclinate nella stessa direzione.

Figure e spiegazioni Tav. VIII. - Fig. IV. Individuo giovane. Fig. IV.2. Sezione dello stesso. Fig. IV.3. Individuo adulto. Fig. IV.4. Asci colle corrispondenti sportule. Stazione.- In febbraio, in mezzo all'erba spontanea dei prati, nell'Istituto Agrario Castelnuovo di Palermo, ai Colli.

Macroscopic description

Ascomata initially hypogeous or semi-hypogeous, more or less globose or pyriform, hollow, substipitate, with a small, rounded or somewhat irregular apical orifice and a circular hollow interior with a hint of introflexions; at maturity partially or fully emerging from the substrate and expanding to 5–10 cm in diameter and 3–7.5 cm in height, becoming distinctly or, less often, indistinctly stipitate and superiorly torn into several large, irregular lobes (lacinias); stipe up to 2.2 × 1.8 cm, usually well-defined, deeply submerged into the soil; old ascocarps occasionally becoming almost flattened or collapsed. **Outer surface** smooth, covered in a fine white bloom and tiny warts (visible under the lens), somewhat humped and lobed,

young specimens ranging from intense, iridescent vinaceous-red, lilac-purple to purple-red or purple-pink, adult specimens becoming vinaceous-red to purple or purple-brown, maintaining the same colour for several days. **Hymenium** smooth, opaque, more or less concolourous with the outer surface or slightly darker, purple, often with a lilac tinge. **Flesh** 2.5 to 4.2 mm thick, fragile, not lactescent, slightly brighter than the outer surface, paler pinkish from the center inwards, tasteless and odorless, but old fruitbodies often developing an acidic smell, somewhat reminiscent of overripe figs or wine caskets.

Microscopic description

Asci cylindrical, 330–380 (–435) × 12–14 (–16) μm, operculate, diffusely amyloid along the entire length but strongly amyloid on the apex (type I), 8-spored, with a conspicuous pleurorhynchous base.

Paraphyses the same length as the asci, cylindrical to clavate, 2.5 to 3.3 μm wide, often enlarged up to 7 (–9) μm at the top, multiseptate, often bifurcated from the lower half and slightly widened at apex, otherwise mitigated; substantial stretches are present especially near the lacinias, where inflated (moniliform) paraphyses can be observed; intracellular pigment slightly yellowish, reddish-brown when viewed at low magnifications. **Ascospores** uniseriate, ellipsoid, (12–) 13.6–16.2 (–18) × (7–) 8.3–11 (–12) μm, Q = 1.35–1.90, Qm = 1.69, smooth, hyaline or subhyaline, medium- to thick-walled, monoguttulate or biguttulate at first, then multiguttulate, with tiny, faintly greenish-yellow guttules, sometimes developing low indistinct warts very late in maturity. **Subhymenium** 150–180 μm thick, in the first section formed by short and small catenule that gradually lengthens to form narrow and elongated hyphae (textura intricata).

Excipulum consisting of a single hyphal-type texture, composed of long bundles of scarcely septate, cylindrical to subglobose hyphae, whose elements measure 25–50 × 12–22 μm. These bundles of hyphae radiate from the subhymenium obliquely, crossing several times and reaching the outer surface, where in some places come together in conical-pyramidal bundles (corresponding to the tiny warts observable macroscopically), with cylindrical or clavate terminal elements, mostly hyaline and not corresponding to the warts, but hyphae lying perpendicular to the hymenium (similar to a cutis). If observed at low magnifications, the entire excipulum presents with a more or less intense amber colour, but the outer part is more coloured by an intracellular pigment, that may arise even in the form of small lumps by staining dark amber brown.

Habitat and ecology

So far restricted to the central and eastern regions of the Mediterranean basin, appearing between January and April, solitary or in small groups, initially developing below the ground and partially surfacing at maturity, in sunny glades, in lawns, among grasses and herbs or on bare calcareous ground, with or without the presence of a tree nearby, but also in typically Mediterranean matorral, near *Cistus* spp., *Pinus halepensis* Mill., *Olea europea* L. and other sclerophyllous vegetation.

Studied collections

CYPRUS: 6th February 2009, Asgata, in litter under *Cistus salvifolius* L. and *Ceratonia siliqua* L., 200 m alt., *leg.* M. Loizides; 16th March 2011 and 31th January 2012, Ypsonas, in field among herbs, 50 m alt., *leg.* M. Loizides, GenBank: MH842196 (ITS rDNA); 30th March 2011, Pera Pedi, in litter under *Cistus* sp., 500 m alt., *leg.* M. Loizides; 19th January 2012, 29th January 2012, 3th February 2012, two different places near Pareklisia, in grassy field, 200 m alt., *leg.* M. Loizides; 15th February 2012, Sfalantziotissa, on bare soil, 100 m alt., *leg.* M. Loizides; 29th January 2013, Limassol, in field on bare soil, 30 m alt., *leg.* M. Loizides; 9th March 2014, Souni, in litter near *Cistus salvifolius* L., *Olea europaea* L., *Pinus brutia* Ten., 450 m alt., *leg.* M. Loizides, GenBank: MH842197 (ITS rDNA). GREECE: 16th March 2013, Pylaia (Thessaloniki), on grass near *Pinus* sp. and *Rosaceae* ssp., 170 m alt., *leg.* A. Athanasiadis, GenBank: MH842198 (ITS rDNA); 16th

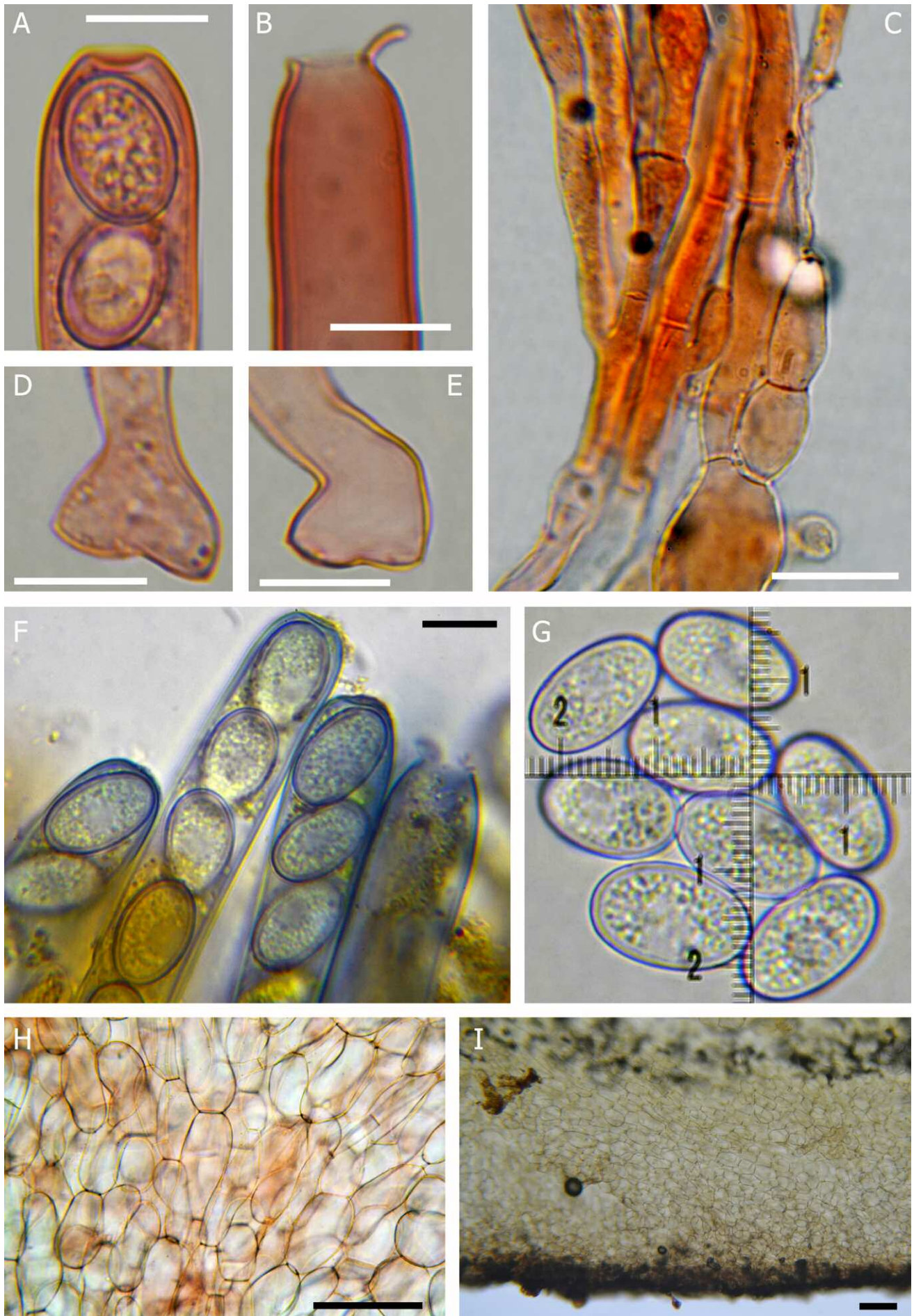


Fig. 2 – *Sarcopeziza sicula*, microcharacters. A, B: Asci in Congo red showing functional operculum; C: Paraphyses in Congo red; D, E: Base of the asci in Congo red; F: Hymenium in Melzer's; G: Ascospores in water; H: Medullary excipulum in Congo red; I: Excipulum in water at low magnification. Scale bar: ABCDEF 10 μm; HI 50 μm. Photos C. Agnello.

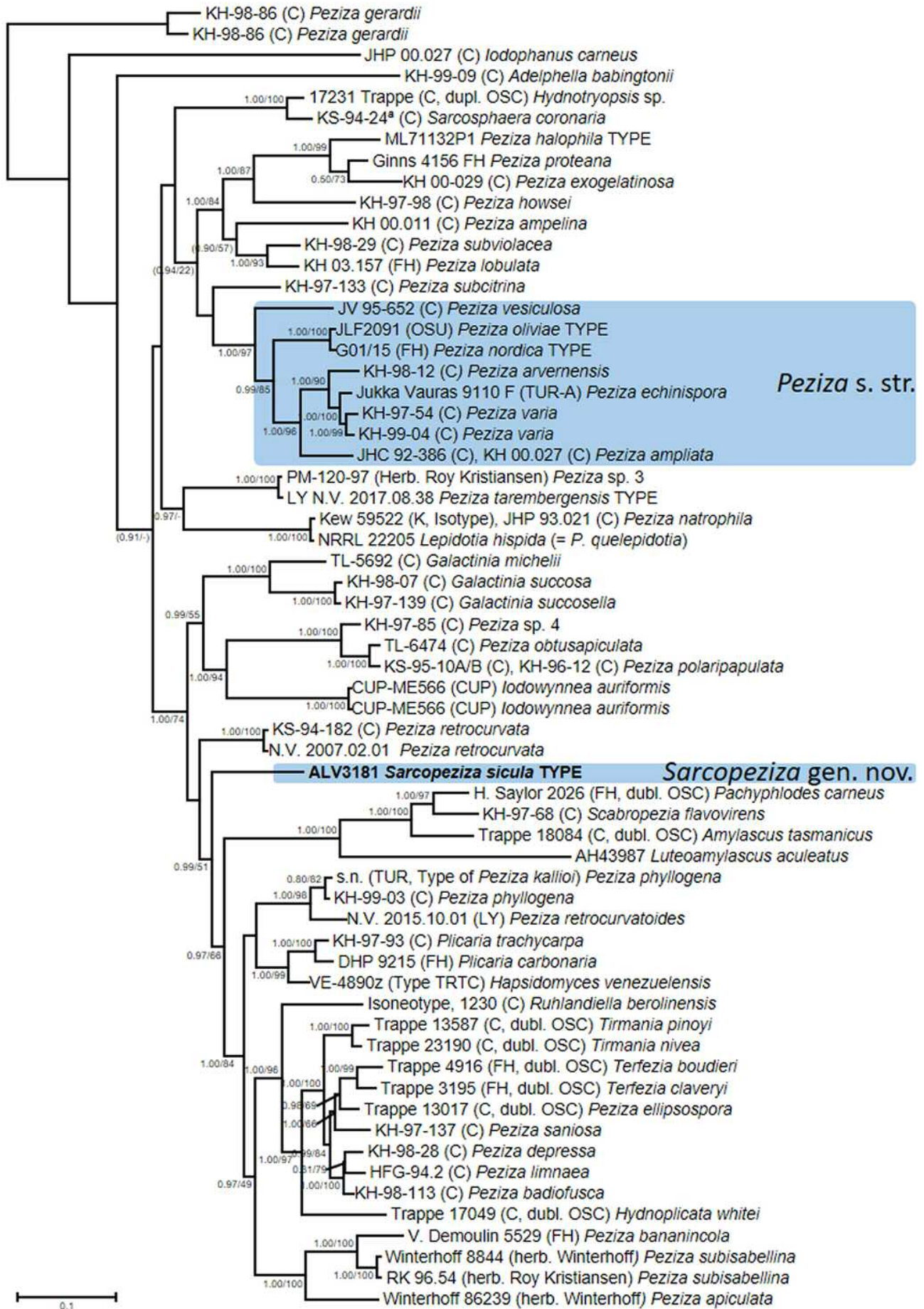


Fig 3 – Bayesian consensus phylogram of *Pezizaceae* obtained in MrBayes from a combined alignment of 28S rDNA, rpb2 and btub datasets. The values next to nodes represent Bayesian posterior probabilities and maximum likelihood bootstrap proportions. Only nodes significantly supported by at least one of these inference methods are shown annotated, although those with subsignificant support are shown in parentheses. The length of the rooting branch was altered for publishing.

March 2013, Kedrinos Lofos (Thessaloniki), on grass in a glade of *Pinus* sp. and *Cupressus sempervirens* L., approx. 350 m alt., leg. I. Parasidis. 3rd March 2014, Pylaia (Thessaloniki), on grass in a glade of *Pyrus amygdaliformis* Vill. little farther *Pinus halepensis* Mill. and *Olea europea* L., 170 m alt., leg. T. Giannakis. ITALY: 7th February 2008, S. Pancrazio Salentino (BR), on grass in a glade of *Pinus halepensis* Mill. and *Olea europea* L., 59 m alt., leg. A. Delle Donne & C. Agnello (*epitypus* MCV 25877), Genbank: MH704524 (ITS), MH704523 (28SnrLSU), MH709116 (rbp2), MH709117 (tef1); 31th January 2009, *ibidem*, leg. A. Delle Donne & C. Agnello.

Additional collections known (not studied by the authors)

GREECE: 18th March, 2013, Pylaia (Thessaloniki), on grass, 180 m alt., leg. I. Parasidis; 21th March 2013, Pylaia (Thessaloniki); on grass, 180 m alt., with near *Pyrus amygdaliformis* Vill., *Olea europea* L., *Pinus* sp., leg. T. Giannakis; 24th February 2014, Peraia, Thessaloniki, on sea level, leg. A. Athanasiadis; 27th February 2014 and 4th March 2014, Pylaia (Thessaloniki), on grass near *Pinus* sp. and *Rosaceae* ssp., 180 m alt., leg. A. Athanasiadis; 3rd March 2014, Pylaia (Thessaloniki), on grass, 180 m alt., leg. Z. Antonopoulos; 8th April, 2014, Pylaia (Thessaloniki), on grass, 100 m alt., near *Pyrus amygdaliformis* Vill., leg. Z. Antonopoulos; 27th January, 2017 and 28th February 2017, Ierissos Halkidiki, on sea level, on grass, leg. G. Psemmas. ISRAEL: 10th February 2006, Bar'am Forest, approx. 100 m alt., leg. R. Kuznetsov; 1st March 2008, Ghilboa Forest, approx. 500 m alt., leg. Z. Shafranov. ITALY: 15th March 2013, Mojo Alcantara (ME), on clay soil with (not neighbors) *Pyrus communis* L. and *Olea europea* L., approx. 500 m alt., leg. D. Principini Cataldo.

Discussion

Following the narrow taxonomic concept of *Peziza* advocated by HANSEN *et al.* (2005) and recently adopted by PFISTER *et al.* (2016), we have here interpreted *Sarcopeziza* as a distinct genus within *Pezizaceae*, and applied the appropriate combination to the only known species of this lineage so far. In many respects, *Sarcopeziza sicula*, along with the morphologically similar *Sarcosphaera coronaria* and species in the *Peziza ammophila* Durieu & Lév. complex, i.e. *P. ammophila*, *P. hellenica* Vizzini, Lantieri & Medardi and *P. oceanica* Vizzini, Lantieri & Medardi (VIZZINI *et al.*, 2016), display transitional features between typically cupulate, epigeous *Pezizaceae* species on one hand, and angiocarpic hypogeous taxa on the other. All these species produce initially hypogeous and enclosed fruiting bodies, which partially emerge at maturity, tearing apically to expose the hymenium and forming acute or irregular lacinias. These macromorphological and developmental similarities were indeed noted by numerous authors in the past, some of whom have placed *P. ammophila* in genus *Sarcosphaera* (SEEVER, 1928; KOTLABA & POUZAR, 1963; GAMUNDI, 1975). However, molecular data place *P. ammophila* in a distant clade from *Sarcosphaera*, sister to *P. vesiculosa*, suggesting that these similarities are likely the result of convergent evolution (HANSEN *et al.*, 2001; VIZZINI *et al.*, 2016).

Several *Pezizaceae* lineages, such as species in the genera *Terfezia* (Tul. & C. Tul.) Tul. & C. Tul., *Tirmania* Chatin, *Pachyphloides* Zobel, or *Luteoamylascus* Cabero, P. Alvarado & G. Moreno (2016), have evolved enclosed, angiocarpic ascumata with tightly packed stereothecia which no longer actively discharge their spores, but instead rely on spore dispersal through mycophagy (FRANK *et al.*, 2006). On the other hand, species such as *Peziza ellipsospora* (Gilkey) Trappe (= *Hydnotrya ellipsospora* Gilkey), *P. whitei* (Gilkey) Trappe (= *Hydnoplicata whitei* Gilkey), or taxa in the genus *Mycoclelandia* (= *Clelandia* Trappe), produce less compact ascumata with loosely arranged pychothecia, which may or may not still have functioning opercula, and could represent intermediate evolutionary stages towards fully sequestrate, truffle-like phenotypes (GILKEY, 1916, 1954; TRAPPE, 1975, 1979; TRAPPE & BEATON, 1984; HANSEN *et al.*, 2001). It would not be unreasonable to assume that *Sarcopeziza sicula*, *Sarcosphaera coronaria*, and species in the *Peziza ammophila* complex, all of which

produce initially enclosed and hypogeous ascumata, but have fully functional opercula — still retaining a forcible spore dispersal (Figs. 2A–B) — may represent early evolutionary forms towards fully sequestrate hypogeous habits. It is interesting to note that *S. coronaria* itself, can sometimes produce fully hypogeous and permanently enclosed ascumata, whose hymenium becomes internally folded and convoluted resembling a pychothecium (TRAPPE, 1975, 1979).

Sequestrate and angiocarpic phenotypes appear to have evolved independently multiple times within *Pezizaceae*, with truffle-like species co-existing with cupulate taxa in at least three of the generic clades identified (clades III, V and VI of HANSEN *et al.*, 2001). Similarly, sarcosphaeroid phenotypes are so far known to occur in at least three generic clades in *Pezizaceae*. Bearing in mind that *Peziza s. str.* also accommodates typically cupulate, epigeous species, it appears that sarcosphaeroid taxa may not only evolve independently across different genera, but also within a genus or an infrageneric clade (VIZZINI *et al.*, 2016). As far as the remaining macromorphological features are concerned, the collections of *S. sicula* from Cyprus appear to be larger in size than Italian collections and previous descriptions of this species, with some specimens reaching 10 cm in diameter at full maturity. Otherwise, the new collections reported here are morphologically identical to the specimens reproduced in the iconotype of INZENZA (1869; see Fig. 1).

The intensity and distribution of the amyloid reaction of the asci has been shown to be an important synapomorphic feature in *Pezizaceae*. HANSEN *et al.* (2001) identified four major types of ascus amyloidity, significantly correlating to different phylogenetic clades: type (I), faintly amyloid over the entire length but intensely and unrestrictedly amyloid over the apex; type (II), faintly amyloid over the entire length but intensely and restrictedly amyloid in the apical arc; type (III), evenly amyloid over the entire length; and type (IV), non-amyloid. *Sarcopeziza sicula* typically shows a faint, diffuse reaction across the entire length of the ascus, with a strong unrestricted reaction over the ascus apex (Fig. 2F), corresponding to type (I). This reaction is very similar to *Sarcosphaera coronaria*, but distinct from species in the *Peziza ammophila* complex, which typically show an intense reaction restricted on the ascus apical arc (type II). We have further noted that, compared to the Italian material (*epitypus* MCV no. 25877), mostly consisting of young specimens, some of the new collections have paraphyses that are not strictly cylindrical but also moniliform and often branching, resembling those of *S. coronaria*. These inflated and somewhat irregularly shaped paraphyses are mostly distributed along the vicinity of the lacinias. Regarding the hyphae of the medullary excipulum, the bundles of cylindrical hyphae there appear more widened, allantoid or subglobose, especially close to the ectal excipulum.

Spore measurements from the Cypriot collections show a slight deviation compared to those from the Italian collections made in 2008/2009, most notably in the width, which can reach 11–12 µm in some cases. This difference is likely due to the fact that a larger number of mature fruiting bodies were available to us and measurements were taken directly from deposits of naturally ejected fully mature spores. Moreover, in very mature specimens, some ascospores were found to develop low isolated warts, difficult to be observed under an optical microscope. These warts are more clearly visible in lactophenol cotton blue, but collections with uniformly smooth ascospores have also been observed and it is possible that this character is not consistently present in all collections. Although the ascospores of the morphologically similar *Sarcosphaera coronaria* are also ornamented with low warts when viewed in Cotton Blue or under SEM, they are typically biguttulate, more narrowly ellipsoid (reaching 18–20 µm in length and rarely exceeding 7–9 µm in width), and rounded or truncated at the poles. The latter species is further distinguished in the field by its externally whitish ascumata (or entirely white in *f. alba*), which are almost always sessile, lacking a discernible stipe.

The collections from Cyprus and Greece reported here, significantly expand the distribution of *Sarcopeziza sicula* to the south-



Fig. 4 – *Sarcopeziza sicula*, various collections; A, B, C: A. Athanasiades (Thessaloniki, GR); D: I. Parisidis (Thessaloniki, GR); E: Z. Antonopulos (Thessaloniki, GR); F: T. Giannakis (Thessaloniki, GR) G: D. Principini Cataldo (Moja Alcantara, IT); H, L: R. Kuznetsov (IL); I: Z. Shafranov (IL).



Fig. 5 – Various collections of *Sarcopeziza sicula* from Cyprus: A: Two ascocarps at different stages of development: On the left a partially surfaced mature ascocarp, on the right (arrow) an immature, hypogeously growing specimen at an early stage of development. B, C: Partially emerging and still angiocarpic fruiting bodies at early stages of maturity. D: Fully mature ascocarp tearing into distinct star-shaped lacinae. Photos M. Loizides.

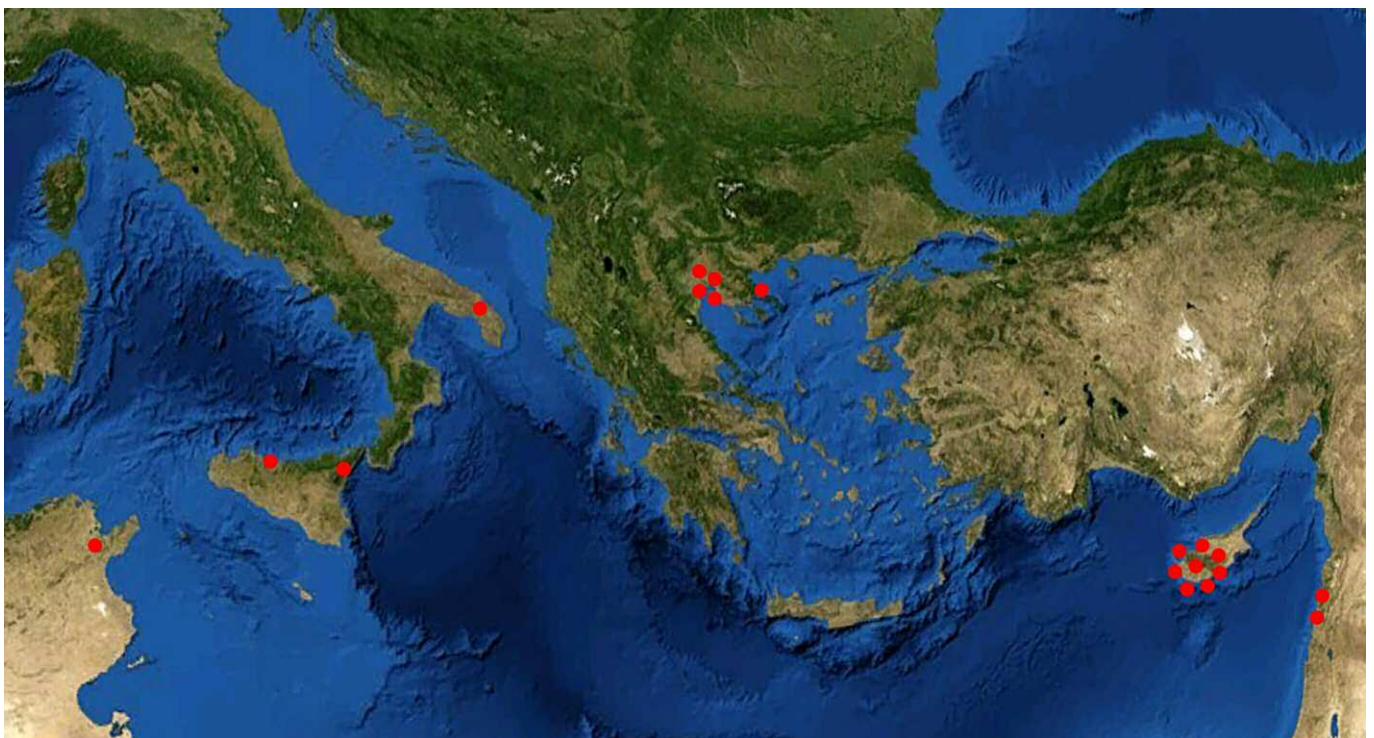


Fig. 6 – *Sarcopeziza sicula*. Distribution map of known localities, from the original collection to date.

eastern boundaries of the Mediterranean basin. This species also appears to be present in Israel [misinterpreted as "*Sarcosphaera coronaria*" in LEWINSOHN (2008), but verified from subsequent collections (R. Kuznetsov, pers. comm.)]. However, it appears to be rare in most of these localities so far, perhaps with the exception of Cyprus where it can be seen in wide range of calcareous habitats, from bare well-drained soil and xeric lowland grasslands, to middle-altitude matorral and forest edges up to 500 m a.s.l. Although its occurrence in treeless environments could be indicative of a saprotrophic lifestyle, ecological niches in *Pezizomycetes* have revealed to be incredibly varied, and several *Pezizales* are now thought to be endophytes or facultative biotrophs, able to switch from a biotrophic ecology associated with living host cells, to a saprotrophic mode of nutrition (ARNOLD *et al.*, 2009; TEDERSOO *et al.*, 2006, 2010, 2013; BAYNES *et al.*, 2012; LOIZIDES *et al.*, 2016). Considering that collections of *S. sicula* under *Cistus* and *Pinus* have also been documented, a more complex lifestyle for this species should not be excluded.

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