

Genea cephalonicae sp. nov. (Ascomycota, Pezizales), a new hypogeous species from Greece

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Ascomycete.org, 8 (3) : 105-110.
Mai 2016
Mise en ligne le 05/05/2016



Abstract: A new species of *Genea* Vittad. from Greek forests of *Abies cephalonica* is proposed based on morphological and genetic studies. Macroscopic images of fresh specimens, as well as observations made under optical microscopes, drawings, a 28S rDNA phylogenetic tree and a small key are provided.

Keywords: *Abies cephalonica*, Europe, hypogeous fungi, phylogeny, *Pyronemataceae*, taxonomy.

Περίληψη: Ένα νέο είδος της *Genea* Vittad. από την Ελλάδα προτείνεται με βάση τις μορφολογικές και γενετικές μελέτες. Μακροσκοπικές εικόνες των φρέσκων δειγμάτων, καθώς και παρατηρήσεις που έγιναν κάτω από οπτικά μικροσκόπια, σχέδια, ένα 28S rDNA φυλογενετικό δέντρο και ένα μικρό κλειδί παρέχονται.

Λέξεις κλειδιά: *Abies cephalonica*, Υπόγειοι μύκητες, Ευρώπη, φυλογένεση, ταξινόμηση, *Pyronemataceae*.

Introduction

Abies cephalonica Loudon, the Greek fir, is an endemic plant of Greece. It received its name from the island of Kefallonia, in the Ionian Sea, where it grows native. Many fungal species associated with this putative ectomycorrhizal host have been recorded, and some of them were new taxa (DIAMANDIS & MINTER, 1981; DIAMANDIS & PERLE-ROU, 1983; DIMOU *et al.*, 2008; PANTIDOU, 1980). We recently described a new hypogeous species found under *A. cephalonica*, *Barssia hellenica* Kaounas, Agnello, P. Alvarado & Slavova (KAOUNAS *et al.*, 2015). Therefore, the occurrence of *Genea* sp. samples under this tree represented an interesting finding because of the endemic character of the host plant, and also because the only other known species of *Genea* located under firs was the rare *G. vagans* Mattir. The morphological and phylogenetic analysis of the collections revealed that they are not related with *G. vagans* but represent a new taxon which is described here.

Materials and methods

Morphological study. — The macroscopic study was conducted on fresh and dried specimens. Micromorphological observations were performed under two different trinocular microscopes with plan achromatic lenses (4×, 10×, 40× and 100× oil immersion). Micromorphological features were studied and measured in water mounts. The inamyloid reaction of asci was checked with Melzer's reagent. Cotton blue was used to highlight spore ornamentations,

and Congo red was employed to stain cell walls of different elements. Finally the dimensions of the ascospores were obtained by measuring at least 50 randomly selected ascospores.

Phylogenetic analysis. — Total DNA was extracted from dry specimens and polymerase chain reaction (PCR) was performed following the procedures described in ALVARADO *et al.* (2016). PCR amplification was primed with ITS1F and ITS4 (WHITE *et al.*, 1990; GARDES & BRUNS, 1993) for ITS, and LR0R and LR5 (VILGALYS & HESTER, 1990) for the 28S region of nuclear ribosomal DNA. PCR reactions were performed under a program consisting of a hot start at 95°C for 5 min, followed by 35 cycles at 94°C, 54°C, and 72°C (45, 30, and 45 s, respectively), and a final extension at 72°C for 10 min. PCR products were checked on 1% agarose gels, and positive reactions were sequenced with the same PCR primers. Chromatograms were checked and the low-quality ends trimmed. No internal ambiguities were found.

28S rDNA sequences were first aligned in MEGA 5.0 (TAMURA *et al.*, 2011) software with its Clustal W application and then corrected manually. The aligned loci were loaded in PAUP* 4.0b10 (SWOFFORD, 2001) and subjected to MrModeltest 2.3 (NYLANDER, 2004). Model GTR+Γ was selected. This model was implemented in MrBayes 3.1 (RONQUIST & HUELSENBECK, 2003), where a Bayesian analysis (BPP) was performed (two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until convergence parameters were met after about 430,000 generations, with the standard deviation having fallen below 0.01. The first 25% of trees were discarded as burn-in. A full search for the best-scoring maximum likelihood



Plate 1 – *Genea cephalonicae*. Collection VK4140 (left) and MCVE 29055 (right).

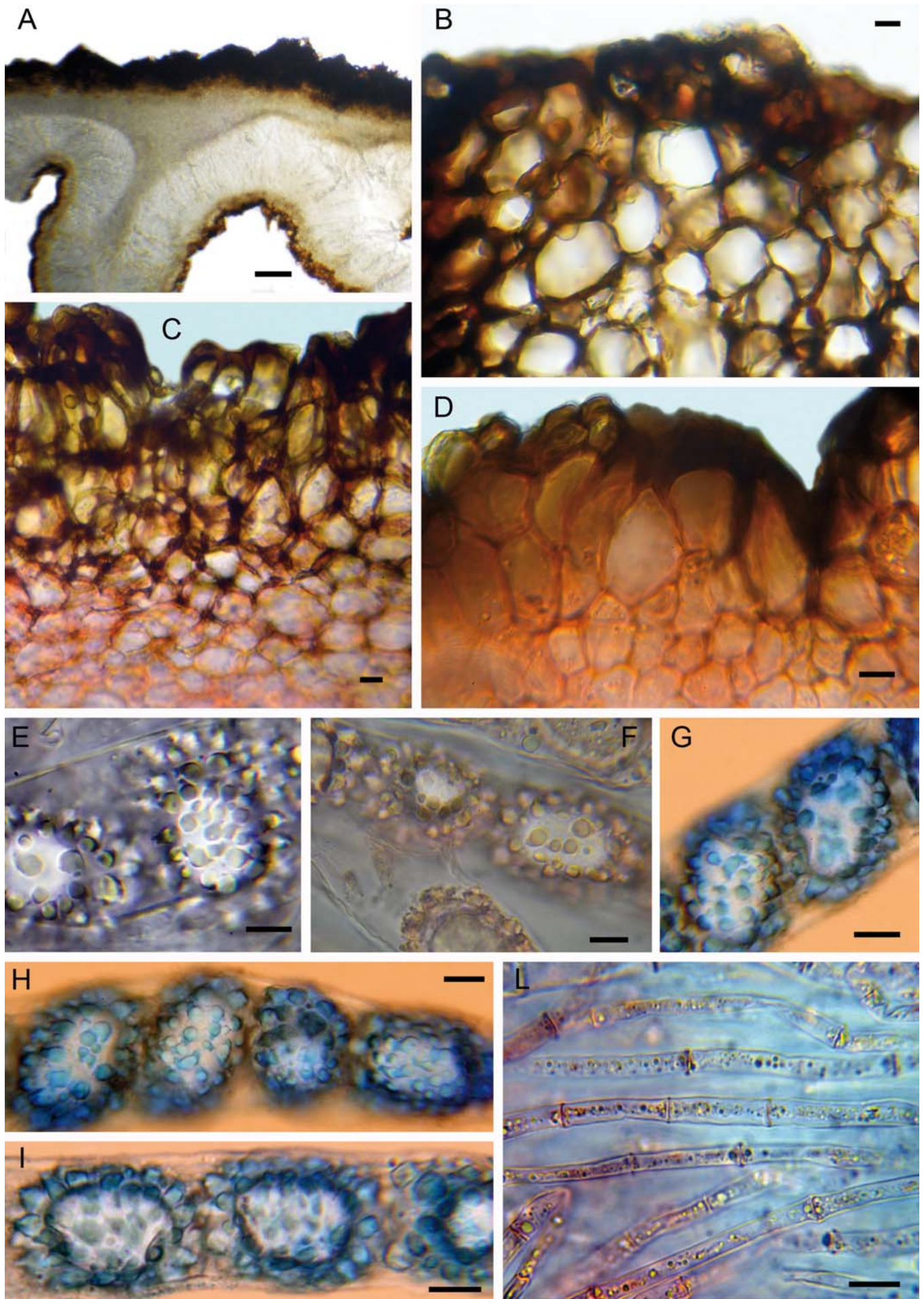


Plate 2 – *Genea cephalonica*. Coll. MCVE 29055. A: Section in water; B: Peridium in water; C-D: Peridium in Congo red; E: Ascospores in water; F: Ascospores in Congo red; G-I: Ascospores in cotton blue; L: Paraphyses in Congo red. Scale bars: A = 100 μ m; B-L = 10 μ m.

tree (ML) was performed in RAxML (STAMATAKIS, 2006) using the standard search algorithm (data partitioned, 2000 bootstrap replications). The significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Taxonomy

Genea cephalonicae Kaounas, Agnello & P. Alvarado, *sp. nov.* – MycoBank MB 816351.

Diagnosis: Differs from the other *Genea* spp. because of its habitat under *Abies cephalonica*, its particular spore ornamentation, and phylogenetic results.

Typification: Holotype here selected MCVE 29055.

Etymology: “*cephalonicae*” refers to *Abies cephalonica* (Greek fir), the dominant tree species where the fungus was discovered.

Ascomata hypogeous, subglobose to elongated, or more or less lobed, measuring 1.5–2 × 1–1.5 cm; external surface black, covered with irregular, minute, densely packed pointed or pyramidal warts and presenting a brown mycelial tuft of hyphae at the base. **Gleba** with sinuous, labyrinth-like folds. **Smell** and **taste** not distinct. **Pedestalium** pseudoparenchymatic, measuring 105–235 µm, composed of dark brown angular external cells 20–30 × 25–40 µm with thick dark walls (2.5–5.0 µm), and an internal layer with subglobose, light brown or yellowish thin-walled cells measuring 20–30 µm in diameter. **Asci** cylindrical, 180–270 × 20–36 µm, containing eight unise-

riate spores, inamyloid. **Paraphyses** hyaline, septate, measuring 3–5 µm in diameter, forming an epithecium. **Ascospores** hyaline, broadly elliptical, measuring (24–)28.5–30(–35) × (15.5–)20–21.5(–26) µm, $Q = (1.2–)1.4(–1.6)$, containing usually two large oil droplets, sometimes just one large and central droplet; spores are ornamented by large warts with rounded tips, 3–5(–6) µm high, rarely digitate, but also sometimes truncated.

Habitat: hypogeous, in small groups under *Abies cephalonica*, in mountainous area (1040 m asl).

Studied collections: GREECE: Mola, mountain of Parnitha, near to Athens, Attica, under *Abies cephalonica*, leg. V. Kaounas, 14.V.2015, VK4140, Genbank 28S rDNA KU933928, ITS KU933927. *Ibidem*, 04.VI.2015, VK4159, MCVE 29055 (holotype), Genbank 28S rDNA KU933930, ITS KU933929.

Discussion

The only other *Genea* species sharing a putatively similar habitat is *G. vagans*, found under “*Abietibus et Fagibus*” (MATTIROLO, 1900). *Genea vagans* is however quite different from the species presented here because of its size, reported by Mattiolo to be smaller than a pea, and spores ornamented with conical warts. CERUTI (1960) examined authentic material from Mattiolo’s herbarium, confirming the original description and providing a summary table that we reproduce here (Pl. 3). MATTIROLO (*op. cit.*) reported that *G. vagans* was found in November in Vallombrosa Forest (Tuscany, Italy) “*sub Abietibus et Fagibus*” but also cited collections found by Bresadola in

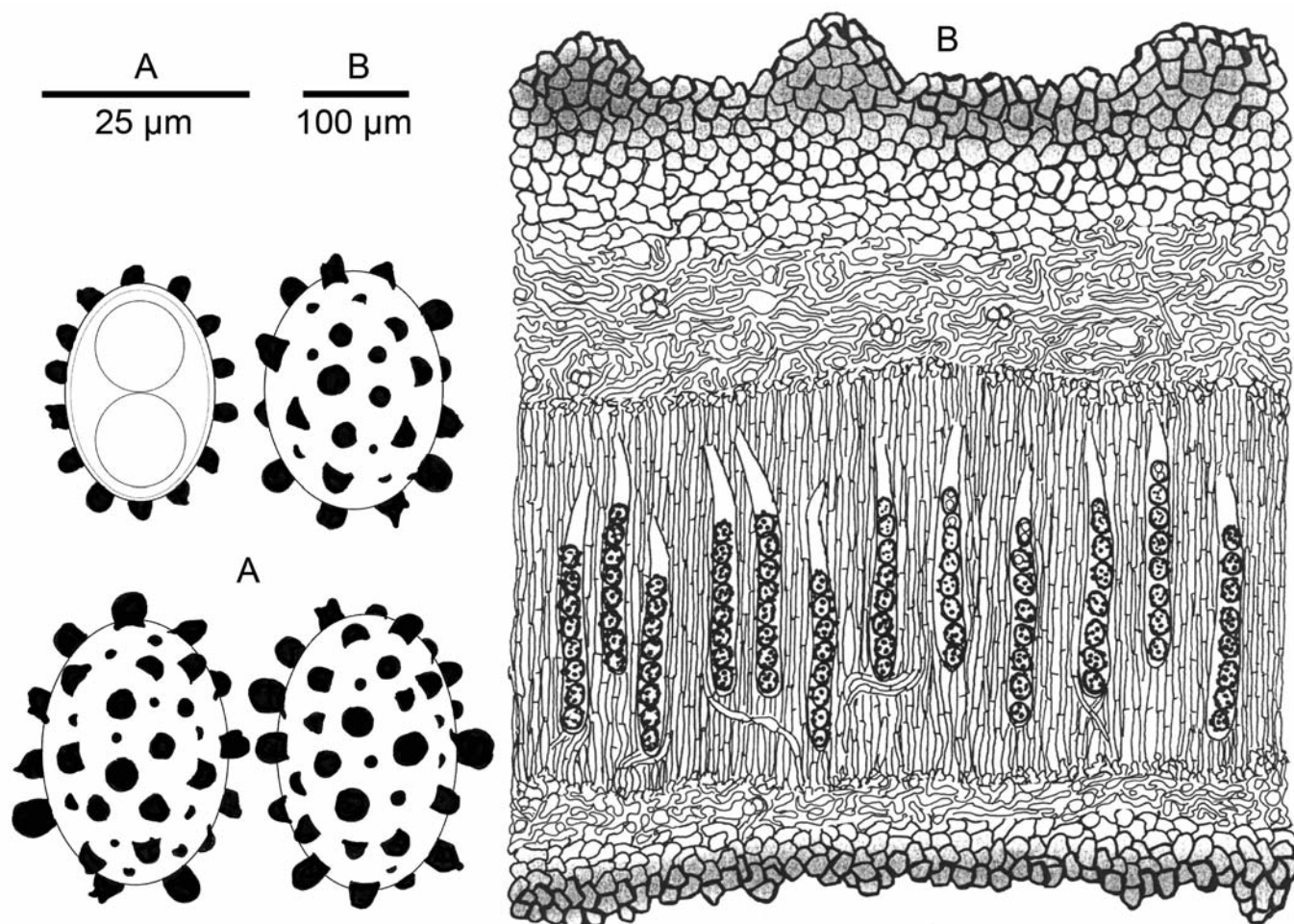










Plate 3 – *G. cephalonicae*. Micro-characters from coll. MCVE 29055. A: Ascospores. B: Section. Drawing: C. Agnello.

A practical morpho-ecological key to the known European species of *Genea* with a dense gleba formed by sinuous-labyrinth-like folds that give it a brain-like appearance

- | | | |
|--|---------------------------------|---|
| Globose, not lobed ascocarp species | 1 |  |
| Subglobose to elongated, irregularly lobed ascocarp | 2 |  |
| 1a. Spores ornamented by flat and large warts, unusual within the genus, similar to plaques, <1 µm high, growing under a variety of host plants | <i>G. lespiaultii</i> |  |
| 1b. Spores ornamented by warts with hemispherical apex, 1–2.5 µm high, growing under <i>Carpinus</i> , <i>Corylus</i> , <i>Fagus</i> and <i>Tilia</i> | <i>G. sphaerica</i> |  |
| 2a. Often irregularly lobed, growing under <i>Quercus ilex</i> , spores ornamented with spiny warts, more rarely conical with roundish top, 1–2.5 µm high | <i>G. lobulata</i> |  |
| 2b. Subglobose or moderately lobed, growing under <i>Q. ilex</i> and <i>Q. pyrenaica</i> , spores ornamented by truncated warts, rarely pointed, occasionally with small digitations at the top measuring 1.5–2.5 (–4) µm high ... | <i>G. pseudobalsleyi</i> |  |
| 2c. Subglobose or irregularly lobed, growing under <i>Abies cephalonica</i> , spores ornamented by rounded but also truncated and rarely digitate warts, 3–5 (–6) µm high | <i>G. cephalonicae</i> |  |
| 2d. Subglobose or lobed, growing under a variety of host plants, including <i>Q. pyrenaica</i> , <i>Q. cerris</i> , and <i>Quercus</i> sp., spores ornamented by conical warts, often digitate at the top, 2.5 µm high | <i>G. compressa</i> |  |

Trentino (Italy) under chestnut, and Bucholz, near Moscow under *Populus* sp., *Betula* sp., *Picea vulgaris*. Recently, Mattirolò's collections of *G. vagans* deposited in the University of Turin, were compared with newly collected specimens (ALVARADO *et al.*, 2016). These were collected under *Quercus ilex*, *Q. pyrenaica*, *Fagus sylvatica*, *Alnus* sp., *Corylus* sp. or *Carpinus* sp. and matched the original reports on spore size and ornamentation.

Some other species of *Genea* resemble macroscopically *G. cephalonicae* because of their large whitish trama plates with sinuous-labyrinth-like folds that give it a brain-like appearance. These are:

- *G. sphaerica* Tul. & C. Tul., an ancient species with a small and globose ascocarp, known to grow under *Carpinus*, *Corylus*, *Fagus* and *Tilia*, has ascospores 28–31 × 21–24 µm excluding ornamentation, ornamented with hemispherical warts 1–2.5 µm high × 1–5 µm diam.

- *G. lobulata* (Mor.-Arr., J. Gomez & Calonge) P. Alvarado & Mor.-Arr., previously considered as a “forma” of *G. sphaerica* was recently raised to the status of an independent species (ALVARADO *et al.*, 2014). It can be found under *Quercus ilex* in Spain, Greece and Cyprus, and has ascospores 24–30 × 20–26 µm, ornamented with spiny warts, more rarely conical with roundish top, measuring 1–2.5 µm high.

- *G. lespiaultii* Corda seems only macroscopically similar but has ascospores ornamented with irregular flat warts, a rare feature not found in any other species of the genus *Genea*.

- *G. pseudobalsleyi* Agnello, Bratek & J. Cabero, growing under *Q. ilex* and *Q. pyrenaica* in Hungary, Italy and Spain, has smaller ascospores than *G. cephalonicae*, 21.7–30.2 × 17.7–26.3 µm, also with a different “Q” and different ornamentations, 1–2.5 (–4) µm high. Furthermore, this species has a pleasant odor very similar to that of *Tuber aestivum* Vittad.

- *G. compressa* Z. Merenyi, J. Cabero & G. Moreno, found under *Q. pyrenaica*, *Q. cerris*, and *Quercus* sp. (also in mixed habitat with *Carpinus betulus* or *Cedrus atlantica*), in Hungary, Morocco and

Spain, has spores 23.5–30.5 × 18–25.5 µm, ornamented by conical warts, often digitate at the top, 2.3–5.3 µm high.

- *G. fageticola* Konstant., J. Cabero & F. Garcia, a recently new species found in Greece growing under *Fagus sylvatica* (ALVARADO *et al.*, 2014), has ascospores with ornamentations somewhat similar to those of *G. cephalonicae*, but is morphologically very different having a single inner chamber without wall projections.

Acknowledgements

We want to express our gratitude to Julio Cabero (Toro, Spain) who showed us many pictures of *G. compressa*, and Nicolas Van Vooren for his valuable suggestions.

References

- ALVARADO P., CABERO J., MORENO G., BRATEK Z., VAN VOOREN N., KAOUNAS V., KONSTANTINIDIS G., AGNELLO C., MERÉNYI Z. & SMITH M.E. 2014. — Species diversity of *Genea* (Ascomycota, Pezizales) in Europe. *Ascomycete.org*, 6 (3): 41–51.
- ALVARADO P., CABERO J., MORENO G., BRATEK Z., VAN VOOREN N., KAOUNAS V., KONSTANTINIDIS G., AGNELLO C., MERÉNYI Z., SMITH M.E., VIZZINI A. & TRAPPE J.M. 2016. — Phylogenetic overview of the genus *Genea* (Pezizales, Ascomycota) with an emphasis on European taxa. *Mycologia*, 108 (2): 441–456.
- CERUTI A. 1960. — *Iconographia Mycologica*. Vol. XXVIII, suppl. II- *Elaphomycetales* et *Tuberales*. Trento, Museo Tridentino di Scienze Naturali-Comitato Onoranze Bresadoliane.
- DIAMANDIS S. & MINTER D. 1981. — A contribution to the mycoflora of Greek fir (*Abies cephalonica* Loud.). Part 1. To Dasos 92: 46–60. [in Greek]



Plate 4 – *Genea vagans*. Mattir., tab. XV, fig. 2, in CERUTI, 1960.



Plate 5 – A view of the forest with *Abies cephalonica*

- DIAMANDIS S. & MINTER D. 1983. — A contribution to the mycoflora of Greek fir (*Abies cephalonica* Loud.). Part 2. Dassiki Erevna 2: 131-136. [in Greek]
- DIMOU D.M., ZERVAKIS G.I. & POLEMIS E. 2008. — Mycodiversity studies in selected ecosystems in Greece: IV. Macrofungi from *Abies cephalonica* forests and other intermixed tree species (Oxya Mt., central Greece). *Mycotaxon*, 104: 39-42.
- GARDES M. & BRUNS T.D. 1993. — ITS primers with enhanced specificity for Basidiomycetes—application to the identification of mycorrhizae and rusts. *Molecular Ecology*, 2 (2): 113-118.
- KAOUNAS V., AGNELLO C., ALVARADO P. & SLAVOVA M. 2015. — *Barssia hel-lenica* sp. nov. (Ascomycota, Pezizales), a new hypogeous species from Greece. *Ascomycete.org*, 7 (5): 213-219.
- MATTIROLO O. 1900. — Elenco dei “funghi hypogaei” raccolti nelle Foreste di Vallombrosa negli anni 1899-1900. *Malpighia*, 14: 247-270.
- NYLANDER J.A.A. 2004. — MrModeltest v2. Program distributed by the author. Uppsala, Evolutionary Biology Centre, Uppsala University.
- PANTIDOU M.E. 1980. — Macro fungi in forests of *Abies cephalonica* in Greece. *Nova Hedwigia*, 32: 709-724.
- RONQUIST F. & HUELSENBECK J.P. 2003. — MrBayes 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19 (12): 1572-1574.
- STAMATAKIS A. 2006. — RAXML-VI-HPC: maximum likelihood based phylogenetic analyses with thousands of taxa and mixed models. *Bioinformatics*, 22 (21): 2688-2690.
- SWOFFORD D.L. 2001. — *PAUP*4.0b10: phylogenetic analysis using parsimony (and other methods)*. Sunderland, Sinauer Associates.
- TAMURA K., PETERSON D., PETERSON N., STECHER G., NEI M. & KUMAR S. 2011. — MEGA5: Molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Molecular Biology and Evolution*, 28 (10): 2731-2739.
- VILGALYS R. & HESTER M. 1990. — Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. *Journal of Bacteriology*, 172 (8): 4238-4246.
- WHITE T.J., BRUNS T., LEE S. & TAYLOR J.W. 1990. — Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: INNIS M.A., GELFAND D.H., SNINSKY J.J. & WHITE T.J. (eds.). *PCR Protocols: A guide to methods and applications*. New York, Academic Press: 315-322.

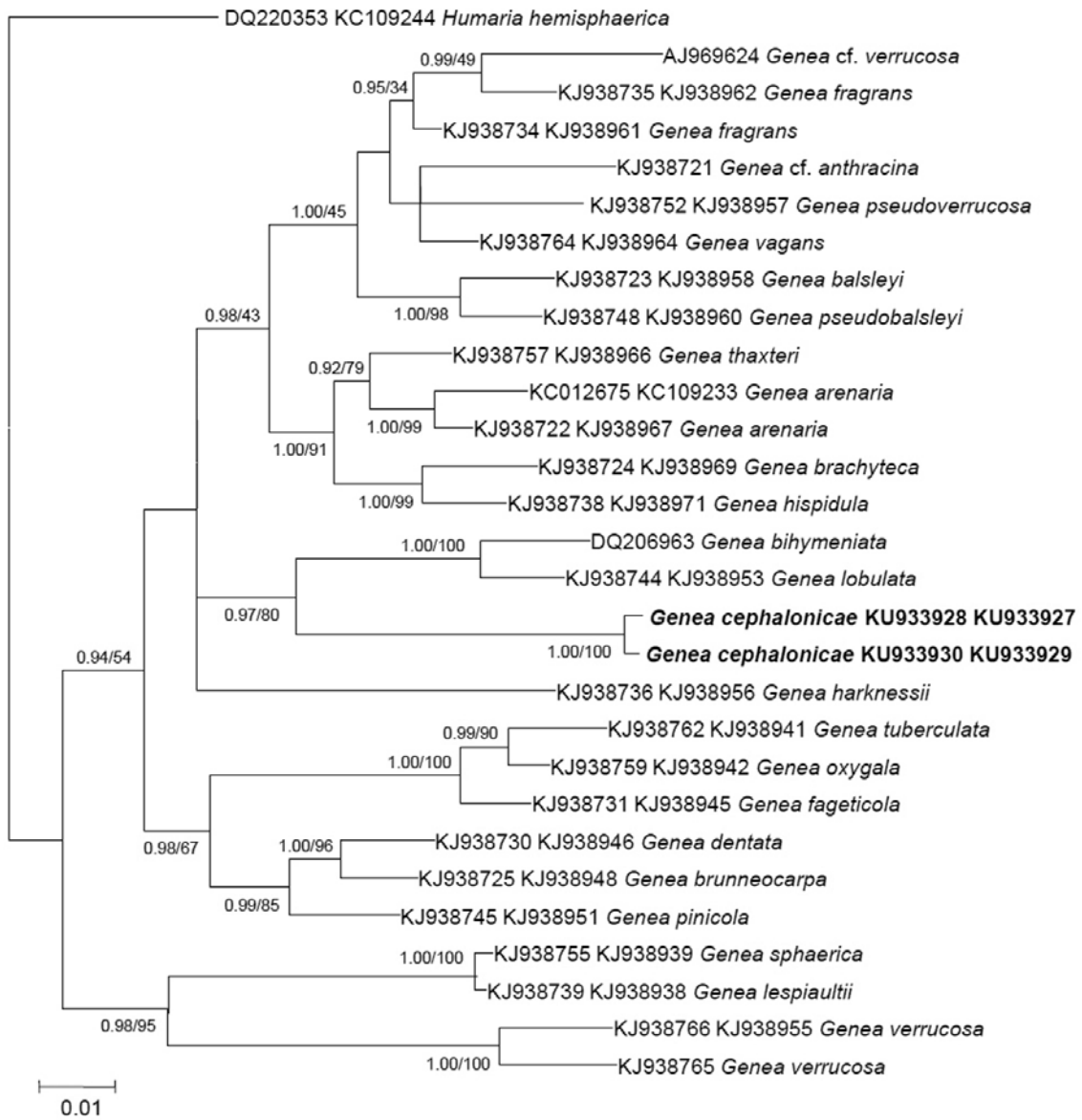


Plate 6 – Consensus phylogram obtained in MrBayes from a combined alignment of 28S rDNA and TEF1- α loci of genus *Genea*. Values next to nodes represent Bayesian PP and maximum likelihood BP. Only nodes supported by >0.95 Bayesian PP or >70% BP are annotated.



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