New data on hypogeous fungi from Greece with special reference to Wakefieldia macrospora (Hymenogastraceae, Agaricales) and Geopora clausa (Pyronemataceae, Pezizales)

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Abstract. This work provides new information about five interesting and uncommon hypogeous fungi from Greece – Balsamia vulgaris, Geopora clausa, Hydnocystis piligera, Sclerogaster compactus and Wakefieldia macrospora. Descriptions of the five species are included based upon Greek collections, accompanied by colour macro- and microphotographs, and molecular data of four of them. On the basis of molecular results, the genus Wakefieldia seems to be closely related to Helveloma in the Hymenogastraceae, while Geopora clausa appears to be related to Geopora in the Pyronemataceae.

Key words: Ascomycetes, Basidiomycetes, Boletales, Geastrales, ITS – LSU

Introduction

Although the first records of hypogeous fungi from Greece date back to the middle of 19th century (Landerer 1858), the knowledge on this diverse ecological group in this country is still relatively scarce (Maire & Politis 1940; Zervakis et al. 1998, 1999; Diamandis & Perlerou 2008; Konstantinidis 2009). It is therefore the first author started collecting hypogeous fungi in 2008, citing rare or unknown species for Greece. (Agnello & Kaounas 2010, 2011). Five uncommon and interesting species are described and illustrated below.

Materials and methods

Hypogeous fungi were collected and identified by the first author, unless otherwise stated. Collections were made in 2008–2011 manually without the aid of dogs. The specimens cited in this paper are preserved and available for consult or revision in the private collection of V. Kaounas, abbreviated as “VK” in the text below, in the Herbarium of the Universidad de Alcalá (AH), in the Herbarium of the Institute of Evolution, University of Haifa (HAI), and in the private collections of A. Montecchi, G. Konstantinidis and Miguel Ángel Ribes (noted as “AM”, “GK”, and “MAR” respectively).

The study of the specimens was conducted both in fresh and dried state. Microscopic study was performed under Nikon Eclipse e100 and Bresser Biolux AL light microscopes. Microscopic slides were mostly prepared in tap water. Congo red was also used as a mounting medium, especially when it was necessary to stain the hyphal walls or the spore ornamentation. Measurements were taken from several slides and 30 spores were measured from each species. The values are given below in the following form: (min–) mean±standard deviation (–max), excluding the ornamentation, where present.

The primary sources used for identification are Hawksworth (1954), Burdsall (1968), Pegler et al. (1993), Astier (1998), Montecchi & Sarasini (2000), Gori (2005) and Agnello (2011), but further essential references are listed under the description of each species.

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Molecular methods followed those described in Moreno et al. (2011). Phylogenetic analysis consisted in a maximum parsimony search in PAUP* 4.0b10 and a Bayesian analysis in MrBayes 3.1. Only values above 70% BP and 90 PP were considered significant. Sequences are stored in public databases under the codes stated in Table 1.

Survey of taxa

**Balsamia vulgaris** Vittad., Monogr. Tuberac.: 30 (1831)

Ascomata hypogeous or semi-hypogeous, tuberiform, sometimes lobed, 1–3 cm in diam, hazel or light brown when young, orange-tinted at maturity; surface covered with darker spherical, angular, pyramidal, or irregular verrucose warts of less than 0.5 mm across. **Peridium** thin, not separated from the gleba, with pseudoparenchymatous structure consisting of polygonal or rounded yellowish elements up to 30 μm in diam. **Gleba** pale white to pale yellow, with numerous small irregular elongated chambers and darker veins; odour at first sweet, then becoming stronger and somewhat unpleasant. **Asci** 8-spored, broadly clavate to ellipsoid or fusiform, stalked, up to 100 × 40 μm. **Ascospores** (22.5–) 27±2.6 (–31) × (9–) 11.6±1.3 (–14) μm, ratio (1.9–) 2.4±0.2 (–2.7) (n = 30), ellipsoid to ellipsoid-cylindrical with rounded apices, hyaline, smooth, usually 3-guttulate with one central and two smaller polar guttules, thin-walled (wall < 1 μm thick), randomly arranged in asci. **Paraphyses** cylindrical, up to 5 μm wide.

Habitat – relatively widespread species that generally occurs in deciduous forests at different altitudes, often under moss in meadows and glades at the edges of woodlands, in autumn and winter.

**Specimens examined.** GREECE, Attika: Katsimidi, under *Cistus creticus* L. and *Quercus ilex* L., ca 650 m, 14 Dec 2008 (VK 604); Schinias, under *Quercus cocifera* L., ca 5 m, 22 Dec 2009 (VK 1290); Parnitha, under *Quercus ithaburensis* subsp. *macrolepis* (Kotschy) Hedge & Yalt., ca 500 m, 16 Nov 2010 (VK 1785); Rafina, under *Quercus cocifera* and *Pinus halepensis* Mill., ca 40 m, 9 Jan 2011 (VK 1916).

Recently reported from Greece by Diamandis & Perlerou (2008).

**Geopora clausa** (Tul. & C. Tul.) Burds., Mycologia 60: 507 (1968)

Ascomata 0.5–2 (–3) cm in diam, hypogeous or semi-hypogeous, somewhat globose, usually more or less lobed or wrinkled, ochraceous or rusty-brown, with thin mycelial tufts seen sometimes at the base; surface covered with dark brown granules; odour strong, fruity. **Peridium** pseudoparenchymatous, composed of inflated or rounded elements of about 20 μm diam. **Gleba** whitish, consisting of a single chamber, often considerably wrinkled and forming a labyrinthoid structure. **Asci** cylindrical, 160–250 × 15–20 μm. **Ascospores** (22–) 23.7±1.0 (–26) × (16–) 17.2±0.7 (–18.5) μm, ratio (1.3–) 1.4±0.1 (–1.5) (n = 30), ellipsoid or ovoid, uniseriate in the asci, smooth, hyaline, guttulate, usually with a single large central guttule. **Paraphyses** cylindrical, septate, 3–6 μm wide at the top.

Habitat – occurs from autumn to spring as hypogeous or sometimes as semi-hypogeous, in sandy soils, especially in coastal pine forests and scrubland in Mediterranean ecosystems.

**Specimens examined.** GREECE, Attika: Schinias, under *Cistus creticus* and *Pinus halepensis*, ca 5 m, 12 Mar 2008, det. G. Konstantinidis & V. Kaounas (VK 313); *idem*, 21 Apr 2008 (VK 379); *idem*, 24 Dec 2008 (VK 630); *idem*, 27 Feb 2009 (VK 752); *idem*, 9 Dec 2009 (VK 1263); *idem*, under *Pinus pinea* L., ca 5 m, 2 Feb 2011 (VK 2029); *idem*, 14 Feb 2011 (VK 2061); *idem*, 11 Mar 2011 (VK 2101); Rafina, under *Pinus halepensis* and *Cistus monspeliensis* L. in sandy soil, ca 20 m, 5 Feb 2011 (VK 2039); *idem*, 9 Feb 2011 (VK 2046). ITALY, Oristano: Orosei, sandy soil under *Juniperus sp.* and *Cistus sp.*, det. A. Montecchi, 17 Feb 1984 (AM 136, AH 39177); SPAIN, Cáceres, Cuestas de Jaraíz de la Vega, 19 Feb 2011, det. C. Gelpi (AH 39181).

**Additional specimens examined.**

**Geopora cooperi** Harkn.: GREECE, Attika: Parnitha, under *Abies cephalonica* Loudon and *Pinus nigra* J.F. Arnold in argillaceous soil, ca 1200 m, 16 Nov 2010, det. V. Kaounas (VK 1783); MOROCCO, Chefchaouen: Rif mountains, under *Abies pinsapo* Boiss. (AH 39089, AH 39106); SPAIN, Guadalajara: Tamajón, under *Q. ilex* and *Juniperus thurifera* L., 24 Nov 1982, det. R. Galán, (AH 9065); unknown origin (AH 9846).

**Geopora foliacea** (Schaeff.) S. Ahmad: SPAIN, Guadalajara (AH 38936, 38937).

There is one earlier reference for the occurrence of this species in Greece (Diamandis & Perlerou 2008). Molecular data of these species show that it is closely related to the main clade of the genus *Geopora* (Tamm et al. 2010), and unrelated to *Hydnocystis* (also see Fig. 21). This confirms the earlier combination made by Burdsall (1968). It should be noticed that the main clade of the genus *Geopora* does not include its type species, *G. cooperi* (Tamm et al. 2010), which seems to be more related to the genus *Picoa* (Sbissi et al. 2011). This could suggest the resurrection of the genus *Sepultaria*, and the respective transfer of *G. clausa*. Two different clades of *G. clausa* are revealed after molecular inference. One of them (VK2101, VK2039, VK2046 and AH39177) seems to match a sequence in public databases coming from an unidentified ectomycorrhizal fungus from southern France, and is somewhat related to several sequences of *Geopora cf. cooperi* from southern Spain. The second one is composed of sample AH39181, and matches the only sequence of *G. clausa* stored in GenBank (JF908766), obtained from an Italian specimen. None of these groups match the other Mediterranean species of *Hydnocystis*, *H. piligera* and *H. beccarii* Mattir. Spore dimensions seem to be over the range reported by Tulasne & Tulasne (1851; 16–19 μm), but still below those of Mattirolo’s *H. beccarii* (24–27 μm).
**Table 1. Specimens used for DNA analysis of ITS/28S nLSU**

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**Figs 3, 12–14, 21**

Ascomata 0.5–3.5 cm in diam, hypogeous, semi-hypogeous or sometimes epigeous, subglobose, slightly lobed, pale yellow, yellowish ochraceous to flesh coloured, hairy-pubescent. Peridium consisting of outer layer composed of up to 500 μm long wavy hairs, raising from inner pseudoparenchymatous layer of rounded or polygonal elements. Gleba thin, cottony-like, lining the chamber; odour fruity. Asci cylindrical, 8-spored, up to 300 × 50 μm. Ascospores (27–) 30.0±1.4 (–32) μm in diam (n = 30), spherical, hyaline, thin-walled, lacking guttules, uniseriate in asci. Paraphyses filamentous, septate, considerably longer than asci, up to 5 μm wide at the top.

Habitat – occurs as hypogeous or semi-hypogeous in sandy soils in scrubland in Mediterranean ecosystems, usually associated with species such as *Cupressus, Pinus, Pistacia, Cistus*, etc.; in Greece possibly associate of *Eucalyptus* sp. and *Olea europaea* L., ca 50 m, 19 Jan 2009 (VK 664); idem, 1 Feb 2010 (VK 1357). ISRAEL, Samaria, Reihan forest, clayish soil under *Pinus* sp., leg. Y. Ur, 19 Feb 2007 (HAI-D-035, AH 39306). ITALY, Oristano: Su Cologone, under *Eucalyptus* sp., det. A. Montecchi, 28 Oct 1984 (AM 134, AH 39178). SPAIN, Islas Baleares, Mallorca, Formentor, Torrent des Estanyol, semihypogeous under *Quercus ilex* with *Chamaerops humilis* L., 8 Dec 2009 (MAR 081209-49, AH 39302); ISLAS BALEARES, Mallorca, Alcudia, Son Serra de Marina, semihypogeous in a moss area under *Juniperus oxycedrus* L., fixed dunes, 6 Dec 2009 (MAR 061209-05, AH 39303). This is the first finding of this uncommon species in Greece. It is similar to *Geopora clausa*, but is easily distinguished on the account of its spherical (not ellipsoid to ovoid) ascospores. The Greek collections fit very well the descriptions in Burdsall (1968), Montecchi & Sarasini (2000), Gori (2005) and Agnello (2011). 28S nLSU data in Alvarado et al. (2011) showed that this species is related to *Stephensia bombycina* (Vittad.) Tul. & C. Tul. and unrelated to *Geopora*. In the present study, ITS data confirmed its status as an independent monospecific genus (Fig. 21).
Fig. 21. Consensus phylogram constructed in Mr. Bayes 3.1 showing the phylogenetic affiliation of the ITS sequences of *Hydnocystis piligera* and *Geopora clausa* obtained in the present study and its closest relatives. Values above nodes represent maximum parsimony bootstrap proportions, while those below nodes are Bayesian posterior probabilities.
Fig. 22. Consensus phylogram constructed in Mr.Bayes 3.1 showing the phylogenetic affiliation of the ITS sequence of *Wakefieldia macrospora* obtained in the present study and its closest relatives. Values above nodes represent maximum parsimony bootstrap proportions, while those below nodes are Bayesian posterior probabilities.

*Sclerogaster compactus* (Tul. & C. Tul.) Sacc., Syll. fung. 11: 170 (1895)  
**Figs** 4, 15–17  
Basidiomata *gasterocarpic*, 0.5–1 (–2) cm in diam, subglobose, smooth, somewhat elastic, initially whitish or yellowish, later dirty yellow. **Peridium** not separable from the gleba, consisting of two layers, the outer filamentous, composed of interwoven hyphae, the inner pseudoparenchymatous, of nearly spherical elements of 10–20 μm in diam. **Gleba** compact, composed of very small compartments (lens required), initially yellowish white to yellow or orange-yellow later; columella absent or rudimentary. **Basidia** not seen.  
**Basidiospores** yellowish or greenish, spherical or sometimes sub spherical, (5–) 6.2±0.7 (–7.5) μm (*n* = 30), verrucose, with *ca* 0.5 μm high cyanophilous warts, and with well seen sterigmal remnants.  
**Habitat** – occurs underground, often in large numbers, beneath decaying leaves or moss in broadleaf, mixed or coniferous forests, from autumn to spring, although Greek collections are made in the winter and spring.
Specimens examined. GREECE, Attika: Schinias, under Pinus pinea, ca 5 m, 21 Apr 2008, det. G. Konstantinidis & V. Kaounas (VK 378); Rafina, under Pinus halepensis and Cistus monspeliensis, ca 20 m, 7 Jan 2010, (VK 1335); idem, 9 May 2011, (VK 2040).

Sclerogaster compactus is somewhat similar to S. gastrosporioides Pilát & Srvcék, but is easily separated by its distinctly smaller spores and the two-layered structure of the peridium. The Greek collections correspond well to the authoritative descriptions in Dodge & Zeller (1934), Hawker (1954), Pegler et al. (1993), Kriegelsteiner (2000), Montecchi & Sarasini (2000), and Gori (2005). This is a rarely recorded species cited here for the first time from Greece (Zeřaví et al. 1998; Diamandis & Perlerou 2008). As it comes out from the available literature it has not been found yet in any of the neighboring countries of the peninsula (see e. g. Jurč et al. 2005; Sesli & Denchev 2008; Denchev & Assyov 2010). 28S nLSU molecular results link those samples to other previous records of S. compactus from Europe (Hosaka & Castellano 2005; Sesli & Denchev 2008; Denchev & Assyov 2010). This is the first finding of this fungus strongly resembles some species of Hymenogaster, but is easily recognized through microscopic examination, which reveals basidiospores characteristically ornamented with prominent conical hilar appendages (Fig. 18). Two poorly known taxa need to be considered in regard to W. macrospora – Sclerogaster porquierollensis Donnadini & G. Riousset and S. rhizopogon Donadini & G. Riousset (Donadini 1979). Both of them have somewhat similarly coloured basidiospores (more or less resembling that of W. macrospora), spores similar in shape, size and ornamentation, and filamentous peridium. Sclerogaster porquierollensis can be distinguished on account of its generally one-spored basidia, although 2-, 3-, and 4-spored basidia have been also observed by the authors. The characteristic features of the remaining species, S. rhizopogon, are considered, by the authors, to be its white to ochraceous peridium and its 2-spored basidia. Both species are regarded as synonyms of W. macrospora by Vidal (1997) and were not considered in later works (Pegler et al. 1993; Montecchi & Sarasini 2000). Caution should be exercised to separate those two entities during identification, if indeed distinct from W. macrospora. On the basis of the present results, Wakefieldia should be considered an independent genus within the family Hymenogastraceae (Agaricales), with a close relationship with the genera Hebeloma, Anamika, Hymenogaster, and Anicolot Natucoria (Fig. 22). The only ITS sequence obtained in this work seems to match another two stored in GenBank coming from environmental samples under Quercus ilex-dominated forest ecosystems in the Mediterranean basin.

This is apparently a very rare species so far known from Belgium, Czech Republic, Germany, Italy, Spain, Switzerland, and the United Kingdom (Hawker 1951, 1954; De Vries 1988; Martin et al. 1993; Pegler et al. 1993; Ludwig & Schnitler 1996; Montecchi & Sarasini 2000; Gori 2005; Riva 2009; Ortega et al. 2010). This is the first finding of this noteworthy species in Greece and apparently also in the Balkan Peninsula, as it comes out from the literature available (see also Diamandis & Perlerou 2008; Sesli & Denchev 2008; Denchev & Assyov 2010). Watling (2008) proposed that this fungus could be mycorrhizal with beech, as it is suggested by the specimens coming from the United Kingdom (Hawker 1951) where it was first collected and described. However, the four Greek collections suggest also a probable association with oaks, in accordance with Martin et al. (1993), Pegler et al. (1993), and Riva (2005) who proposed a wider range of hosts.

Wakefieldia macrospora is the only European species of this small genus previously thought to be closely related to boletes (Corner & Hawker 1953; Watling 2008). Macroscopically this fungus strongly resembles some species of Hymenogaster, but is easily recognized through microscopic examination, which reveals basidiospores characteristically ornamented with prominent conical hilar appendages (Fig. 18). Two poorly known taxa need to be considered in regard to W. macrospora – Sclerogaster porquierollensis Donnadini & G. Riousset and S. rhizopogon Donadini & G. Riousset (Donadini 1979). Both of them have somewhat similarly coloured basidiospores (more or less resembling that of W. macrospora), spores similar in shape, size and ornamentation, and filamentous peridium. Sclerogaster porquierollensis can be distinguished on account of its generally one-spored basidia, although 2-, 3-, and 4-spored basidia have been also observed by the authors. The characteristic features of the remaining species, S. rhizopogon, are considered, by the authors, to be its white to ochraceous peridium and its 2-spored basidia. Both species are regarded as synonyms of W. macrospora by Vidal (1997) and were not considered in later works (Pegler et al. 1993; Montecchi & Sarasini 2000). Caution should be exercised to separate those two entities during identification, if indeed distinct from W. macrospora. On the basis of the present results, Wakefieldia should be considered an independent genus within the family Hymenogastraceae (Agaricales), with a close relationship with the genera Hebeloma, Anamika, Hymenogaster, and Anicolot Natucoria (Fig. 22). The only ITS sequence obtained in this work seems to match another two stored in GenBank coming from environmental samples under Quercus ilex-dominated forest ecosystems in the Mediterranean basin.

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