Mycologia, 108(2), 2016, pp. 441–456. DOI: 10.3852/15-199

Phylogenetic overview of the genus *Genea* (Pezizales, Ascomycota) with an emphasis on European taxa

Pablo Alvarado[1]
ALVALAB, La Rochela 47, E-39012 Santander, Spain

Julio Cabero
C/ Barrio del Carmen 6, E-49800 Toro, Zamora, Spain

Gabriel Moreno
Departamento de Ciencias de la Vida (Botanica), Universidad de Alcalá, Facultad de Biología, E-28871 Alcalá de Henares, Spain

Zoltán Bratek
Department of Plant Physiology and Molecular Plant Biology, ELTE University, Budapest, Hungary

Nicolas van Vooren
36 rue de la Garde, F-69005 Lyon, France

Vasileios Kaounas
Sokratous 40, Artemis Attika, TK-19016, Greece

Giorgos Konstantinidis
Ag. Kosma 25, 51100 Grevena, Greece

Carlo Agnello
Via Antonio Gramsci 11 – I-72023 Mesagne, Italy

Zsolt Merényi
Department of Plant Physiology and Molecular Plant Biology, ELTE University, Budapest, Hungary

Matthew E. Smith
Department of Plant Pathology, University of Florida, Gainesville, Florida 32611

Alfredo Vizzini
Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Viale P.A. Mattioli 25, I-10125, Torino, Italy

James M. Trappe
Department of Forest Ecosystems and Society, Oregon State University, Corvallis, Oregon 97331

Abstract: We constructed a comprehensive phylogeny of the genus *Genea*, with new molecular data from samples collected in several countries in temperate and Mediterranean Europe, as well as North America. Type specimens and authentic material of most species were examined to support identifications. The molecular identity of the most common species in *Genea* was compared with nuc rDNA internal transcribed spacer (ITS), D1-D2 domains of 28S nuc rDNA (28S rDNA) and translation elongation factor 1-α gene (*TEF*1) profiles of 10 recently proposed taxa, *G. brunneocarpa*, *G. compressa*, *G. dentata*, *G. fageticola*, *G. lobulata*, *G. oxygala*, *G. pinicola*, *G. pseudobalsleyi*, *G. pseudoverrucosa* and *G. tuberculata*, supporting their status as distinct species. *Genea mexicana* and *G. thaxteri* on the one hand and *G. sphaerica* and *G. lespiaultii* on the other are closely related. Multiple lineages were recorded for *G. verrucosa* and *G. fragrans*, but we found no morphological traits to discriminate among them, so we tentatively interpreted them as cryptic species. A key to species of the genus *Genea* is provided to facilitate identification. We provide macroscopic images of fresh specimens and of representative spores of most species. Finally, we conducted a molecular analysis of the divergence time for *Genea* and discuss the implications of our results.

Key words: hypogeous, Mediterranean, phylogeny, Pyronemataceae, taxonomy, truffle fungi

Introduction

Carlo Vittadini (1831) proposed the hypogeous ascomycete genus *Genea* Vittad. dedicated to zoologist Dr Joseph Gené. It is one of the more common truffle genera in the Mediterranean basin, although it receives considerably less attention than the esteemed true truffles in *Tuber* P. Micheli ex F.H. Wigg. *Genea* accommodates species with odoriferous hypogeous ascomata that are more or less lobed and have a basal tuft of hyphae that attaches the ascomata to the substrate. The inamyloid, uniseriate, cylindrical asci typically contain eight verrucose spores and are arranged in an organized hymenium (ptycothecium) and protected by an epithecium that usually resembles the peridium. Trappe (1979) considered *Genea* as the type genus of the family Geneaceae, but it later was transferred to the Pyronemataceae on the basis of both morphological and molecular evidence (Pfister 1984, Smith et al. 2006, Perry et al. 2007, Hansen et al. 2013). There are approximately 35 validly described species currently included in *Genea*, and several varieties justified by different spore dimensions (Gross 1992, 1996).

The type species, *Genea verrucosa* Vittad., was described with hollow ascomata covered with minute black warts on the surfaces of both the peridium and epithecium (Vittadini 1831). Originally it was reported to have spherical spores, but they later were described from authentic material as ellipsoidal, verrucose and

Submitted 23 Jul 2015; accepted for publication 11 Nov 2015.
[1] Corresponding author. E-mail: pablo.alvarado@gmail.com

< 32 μm diam by Berkeley and Broome (1846) and Tulasne and Tulasne (1851). Mattirolo (1900a) described the ascospores of this species as ellipsoidal, 27–30 μm long × 21 μm wide, and ornamented with small, regularly spaced, conical or hemisphaerical protuberances. He also discussed the great color variability of *G. verrucosa*. He described it as ranging from tan to dark brown to rarely black and suggested that color was influenced by developmental stage of the ascomata as well as collection site. Finally, he reported that most original specimens of *G. verrucosa* from Vittadini were covered with pyramidal warts similar to those of *Tuber aestivum*.

Vittadini (1831) also described *G. papillosa* Vittad. as forming smaller, more reddish ascomata covered with papillae. Mattirolo (1900a) considered *G. papillosa* a variant of *G. verrucosa* differing only in the reddish tones of the peridium and proposed the variety *G. verrucosa* var. *badia* Mattir. Zobel (Corda 1854) proposed *G. kunzeana* Zobel, because it was brown and had subglobose and lobed but not wrinkled ascoma. However, Mattirolo (1900b) synonymized *G. kunzeana* with *G. verrucosa* var. *badia* and *G. papillosa* because these taxa have similar spore sizes and overall peridium color. Several authors (Fischer 1897, Hawker 1954, Ceruti 1960) also considered *G. perlata* Corda (Corda 1854) a likely synonym of *G. verrucosa*, because the former differs only by having an irregular ascoma, a feature observed also in *G. verrucosa* by other authors (Tulasne and Tulasne 1851, Mattirolo 1900b). Moreno-Arroyo et al. (1998b) described another species putatively related to *G. verrucosa* from southern Spain, *G. subbaetica* Moreno-Arroyo, Gómez & Calonge. The spores are similar to those of *G. verrucosa*, but *G. subbaetica* has smaller cells in the outer peridial layer and is collected in early winter, while *G. verrucosa* is usually found in spring or early summer.

Wallroth (1833) described some specimens of a hypogeous fungus in Germany as *Hydnocaryon fragrans* Wallr. This was considered a synonym of *G. verrucosa* by Dietrich and Klotzsch (1839) but soon recognized as an independent taxon by Berkeley and Broome (1846) as *G. klotzschii* Berk. & Broome. They incorrectly gave the species epithet *klotzschii* priority over *fragrans*, later corrected as *G. fragrans* (Wallr.) Paoletti by Saccardo (1889). This species was distinguished by subglobose spores that are larger than those of *G. verrucosa* (Berkeley and Broome 1846, Corda 1854, Fischer 1897), and ornamented with block-like or pyramidal warts (Hawker 1954). Another species with fairly large spores, *Genea vagans* Mattir., was proposed by Mattirolo (1900a). This species forms small, round ascomata with a black verrucose peridium. The spores are ellipsoidal, 35 × 27 μm, and ornamented with big conical warts that tend to coalesce at their bases (Ceruti 1960). *Genea vagans* is reportedly found in autumn under *Abies*, *Fagus*, and putatively *Castanea* (Mattirolo 1900b). Velenovský (1922) proposed *G. neuwirthi* Velen. a unique species based on its smooth brownish peridium. This species is reported to have ascomata with a single hollow cavity, spores 35–40 μm and ornamented with prominent thick warts that have blunt, rounded edges.

Berkeley and Broome (1846) also published a description of some *G. papillosa* specimens from the UK that were quite different from those originally described by Vittadini (1831). These later were recognized as a new species by Tulasne and Tulasne (1851) under the name *G. hispidula* Berk. ex Tul. & C. Tul. Tulasne and Tulasne (1851) describe *G. hispidula* as having small, globose to flattened ascomata with a single inner cavity, a basal tuft of reddish mycelium, a peridium covered by pyramidal warts that are smaller than those of *G. verrucosa*, and an epithecium with minute blackish warts. Spores of *G. hispidula* are described as ellipsoidal, 38–42 × 32 μm, and ornamented with crowded, obtuse to roundish warts. *Genea hispidula* is thought to be a symbiont of *Fagus* and *Castanea* trees in France, Britain and Germany (Tulasne and Tulasne 1851, Fischer 1897, Hawker 1954). Corda (1854) proposed a second species with hairy peridium from the Czech Republic, *G. pulchra* Corda. This species differed from *G. hispidula* because of its smaller spores (20–28 × 15–24 μm), spore ornamentation composed of minute or subacute warts and a pseudoparenchymatic peridium with scattered lacunae (locules inside the ascomata that are lined with a palisade of hymenium).

Another species was described by Tulasne and Tulasne (1851) from France as *G. sphaerica* Tul. & C. Tul. and later reported from several European countries (Hesse 1891, Vacek 1951, Hawker 1954). As suggested by the name this species forms almost perfectly sphaerical, medium-sized ascomata that are blackish and in which the peridium and epithecium are covered with minute polygonal warts. Internally it has labyrinth-like chambers and a characteristically white trama. Spores are ellipsoidal, ornamented with obtuse roundish warts and are larger than those of *G. verrucosa*. Mattirolo (1903) described *G. sphaerica* f. *sporis spinuloso-tuberculatis*, a Mediterranean variety of *G. sphaerica* with spores ornamented with spiney-tuberculate warts. A more or less similar feature also was observed by Moreno-Arroyo et al. (1998a) in their *G. sphaerica* f. *lobulata* Mor.-Arr., J. Gómez & Calonge described from southern Spain as a lobed variety of *G. sphaerica*. The species *G. lespiaultii* Corda (Corda 1854) is macroscopically similar to *G. sphaerica* (Mattirolo 1903) but has spores with unusual, irregularly flat warts.

Although *Genea* is a common and frequently collected genus of truffles across both Europe and North

America (Montecchi and Sarasini 2000; Gori 2005; Smith et al. 2006, 2007; Trappe et al. 2007, 2009; Guevara-Guerrero et al. 2012), there has been no modern synthesis based on both morphological and molecular data. The only available molecular phylogenies thus far were based on partial 28S rDNA and included only *Genea* from the western USA (Smith et al 2006, Perry et al. 2007). Ten new *Genea* species were proposed to accommodate several groups of specimens with shared morphological and ecological features collected from European countries (Alvarado et al. 2014). Here we compare these recently described taxa with previously described species based on molecular data to support their taxonomic status. The purpose of this work is to delimit the phylogenetic species within *Genea*, characterize the common taxa in Europe and determine the phylogenetic relationships within the genus.

MATERIALS AND METHODS

Fungal samples.—More than one hundred *Genea* specimens have been studied through morphological or molecular methods or both. European collections are preserved in the herbaria of the Universidad de Alcalá (AH), Hungarian Natural History Museum and Budapest (BP), whereas North American taxa are deposited at the University of Florida (FLAS). Original codes from personal herbaria also are provided (SUPPLEMENTARY TABLE I).

Morphological studies.—Samples were morphologically identified with the aid of available literature (Gilkey 1954, Hawker 1954, Ceruti 1960, Montecchi and Sarasini 2000). Newly collected specimens also were compared with authentic material of common European *Genea* species stored at the University of Turin (TO) as part of Mattirolo's collections. These were sent to Mattirolo from the personal herbaria of the species authors (Mattirolo 1900b). Authentic specimens included samples of *G. verrucosa*, *G. papillosa*, *G. sphaerica*, *G. sphaerica* f. *sporis spinuloso-reticulatis* and *G. vagans*. The type specimen of *G. hispidula* and *G. klotzschii* were obtained from K (Royal Botanical Gardens, Kew, UK), and compared with new specimens. Authentic specimens of American species also were compared with the newly analysed samples. Original material came from TO, BERN (University of Bern, Switzerland) and OSC (Oregon State University, USA). Finally, a comprehensive descriptive work of authentic material of common European and American *Genea* species by J.M. Trappe (unpubl) was used as a guide for comparisons and identifications.

Microscopic images were taken with an Olympus BH2-BHS 100W camera with Apo optical. Helicon Focus software (Helicon Soft. Ltd.) was used to blend focused areas of multiple images. Spores were measured with Piximètre software (Alain Henriot) and Mycometre 2.01 (Georges Fannechère). Microscopic study was performed in water, lactophenol cotton blue, Trypan blue or Congo red-floxine. Scanning electron microscopy (SEM) was performed at University of Alcalá (Spain) with a Zeiss DSM-950. Ascospore sizes exclude spore ornamentation and both ascospore sizes and spore ornament sizes are presented as height × width.

DNA processing and phylogenetic analyses.—DNA extraction and PCR amplification were performed as described by Alvarado et al. (2012). Primers ITS1F and ITS4 (White et al. 1990, Gardes and Bruns 1993) were used for the ITS region; primers LR0R and LR5 (Vilgalys and Hester, 1990) were used for the 28S rDNA ribosomal region (28S rDNA) and EF1-983F and EF1-1567R (Rehner and Buckley 2005) for the translation elongation factor 1α (*TEF*1) gene, for both PCR and sequencing. Sequences were edited for errors in MEGA 5 (Tamura et al. 2011).

Sequence alignment and phylogenetic analyses.—Sequences were aligned with the most similar sequences in GenBank identified through BLASTn searches (Altschul et al. 1997). Two alignments were constructed: 192 ITS sequences and a combined 28S rDNA-*TEF*1 alignment including sequences from 65 specimens. Homologous sequences of *Genea* were retrieved from Smith et al. (2006), Tedersoo et al. (2006), Perry et al. (2007), Guevara-Guerrero et al. (2012), Hansen et al. (2013) and Osmundson et al. (2013). Sequences first were aligned in MEGA 5 (Tamura et al. 2011) with Clustal W (Higgins et al. 1994) and edited manually. The ITS alignment contained insertions in some species, but these regions were excluded from the final anlyses. Alignments are available in TreeBase (ID18402).

Phylogenetic analyses follow those of Alvarado et al. (2012). Aligned loci were subjected to MrModeltest 2.3 (Nylander 2004) in PAUP*4.0b10. Bayesian analysis was performed with optimal models in MrBayes 3.1 (Ronquist and Huelsenbeck 2003) with ITS1-5.8S-ITS2 data partitioned, two simultaneous runs, six chains, temperature set to 0.2, and sampling every 100th generation until convergence parameters were met after about 5 550 000 (ITS) and 1 390 000 generations (28S rDNA – *TEF*1). The first 25% trees were discarded as burn-in. Last a full query for the best-scoring maximum likelihood tree was performed in RAxML (Stamatakis 2006) using the standard search algorithm (ITS1-5.8S-ITS2 data partitioned, 2000 bootstrap replications). Support values were considered significant when bootstrap (BP) values were above 70% and posterior probability (PP) values were above 0.95.

Estimation of divergence times.—*Genea* divergence times were estimated with BEAST 1.8 (Drummond et al. 2012). Because no fossil records or previous studies of dating time are available for Pyronemataceae, we employed a secondary calibration approach according to Prieto and Wedin (2013) and Bonito et al. (2013). Molecular clock analysis of the ITS region was rejected because ITS was too divergent. Thus only the 28S rDNA dataset was analyzed. The jModeltest (Posada 2008) identified GTR+G (Rodríguez et al. 1990) as the best fit to our data. We created two monophyletic taxon sets; the first contains all Pezizomycetes taxa except *Orbilia* sp.; the second contains two Tuberaceae sequences (*T. aestivum* and *Choiromyces meandriformis*). Analyses were run under an uncorrelated lognormal relaxed molecular clock, setting the tree prior to the birth-death speciation

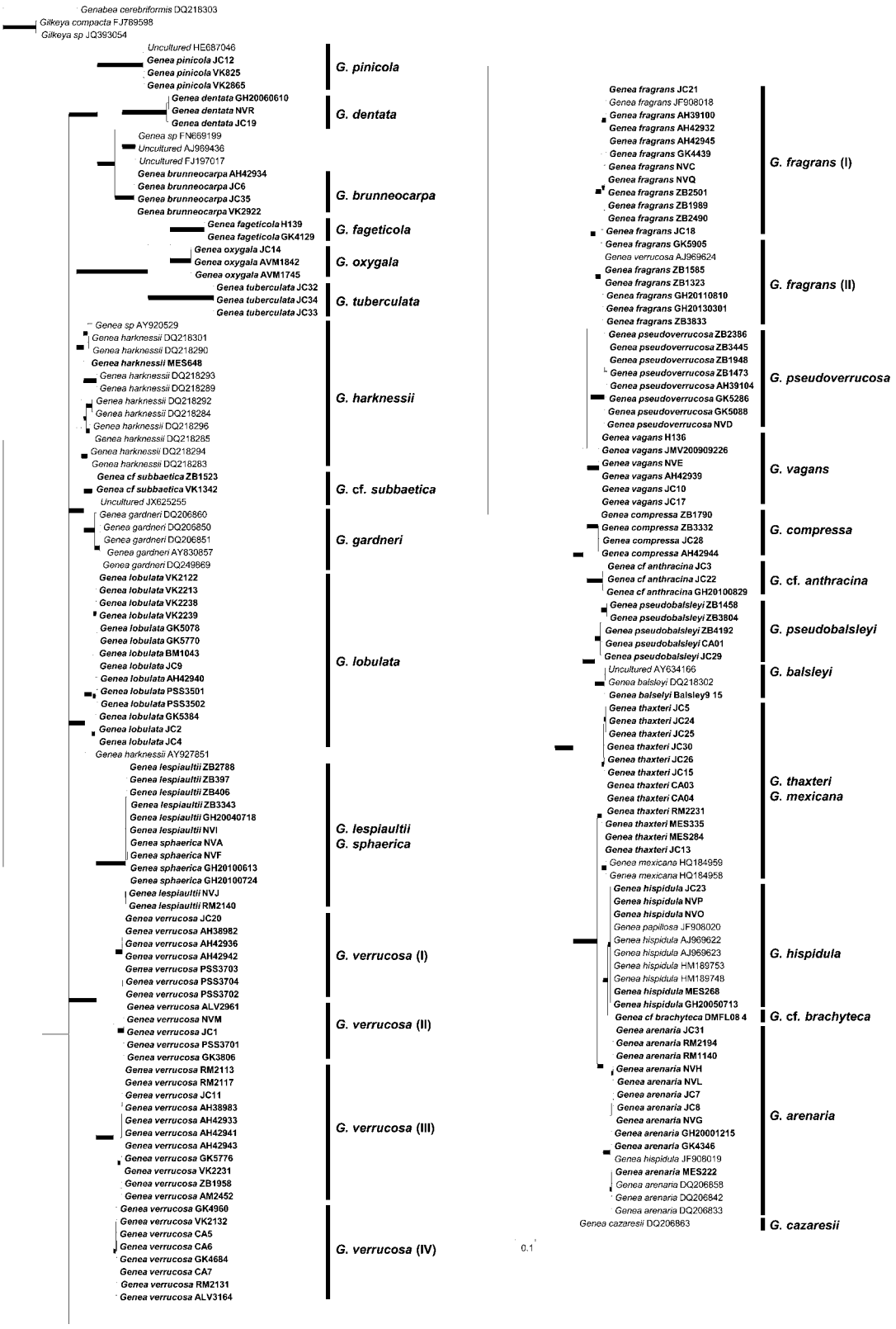

FIG. 1. 50% majority rule consensus ITS rDNA phylogram of the genus *Genea* obtained in MrBayes from 20 550 sampled trees. Nodes supported by >0.95 Bayesian PP and >70% ML BP are shown with boldface bars.

process. We set a normal prior distribution for the node of Pezizomycetes group (mean = 317 Mya, SD = 37.0 Mya), as well as for the node of Tuberaceae (mean = 156.9 Mya, SD = 9.0 Mya). A time-calibrated maximum clade credibility (MCC) tree was reconstructed by running the MCMC simulation for 10 million generations, sampling trees every 1000 generations and discarding the first 50% sampled trees as burn-in. The effective sample sizes were checked with the program TRACER 1.5 (Rambaut and Drummond 2009).

RESULTS

The phylogenetic clades that were recovered based on ITS rDNA (FIG. 1) and 28S rDNA - *TEF*1 analysis (FIG. 2) matched the morphological species concept of circumscribed taxa in the genus *Genea*. Morphologically (FIGS. 3–6) LM and SEM microscopy confirmed the spore descriptions that have been reported for recently described species (Alvarado et al. 2014). Samples from different species and countries (Greece, Spain and Germany) were colonized by a basidiomycete with a 100% ITS match to *Infundibulicybe geotropa* (Bull.) Harmaja (ALV4344, GenBank KT122792, KT122793). Specimens of *G. hispidula*, *G. arenaria*, *G. fragrans*, *G. balsleyi* and *G. harknessii* matched sequences with these names already in public databases. Several taxa formed monophyletic groups with significantly distinct subclades. The high genetic distances of some subclades suggest the presence of cryptic species within the morphological species concepts, e.g. in *G. verrucosa* and *G. fragrans*. Sequences of *G. sphaerica* and *G. lespiaultii* belong to a single monophyletic group and differ by only 6/570 bp in ITS, mainly because of two repetitive insertions in *G. sphaerica*. 28S rDNA sequences were almost identical between *G. lespiaultii* and *G. sphaerica* (FIG. 2), with only scattered point mutations, while these species differed in only one nucleotide in the *TEF*1 gene.

Many unidentified light brown European specimens as well as several identified as *G. verrucosa* f. *badia* had ITS sequences clustered with the North American taxa *G. thaxteri* Gilkey and *G. mexicana* Guevara, Göker & Stielow (Cázares et al. 1992, Guevara-Guerrero et al. 2012), indicating that this clade is found in Europe and North America. Three European collections were tentatively identified as the North American taxon *G. anthracina* based on morphology (Stewart and Heblack 1979) and comparison with ITS rDNA data obtained from *G. anthracina* isotype (OSC 39,482). The sequence (KT950256) was clean and had a clear relationship with those obtained from European specimens, although it probably represents an independent lineage. The only specimen identified as *G. brachytheca* revealed a significant genetic relationship with *G. hispidula* (FIGS. 1, 2) but is here maintained as an independent taxon until further analysis. We also

FIG. 2. 50% majority rule consensus 28S rDNA-*TEF*1 phylogram of the genus *Genea* obtained in MrBayes from 10 425 sampled trees. Nodes supported by >0.95 Bayesian PP and >70% ML BP are shown with boldface bars and major clades are labeled A through G. Nodes supported by ITS inference, but not in 28S rDNA-*TEF*1 analysis are annotated shown as labels with the actual Bayesian posterior probabilities (left) and ML bootstrap proportions (right).

generated the first sequences from specimens of *G. vagans* (Mattirolo 1900b, Ceruti 1960) suggesting that this taxon is monophyletic. In contrast, specimens identified as *G. harknessii* were not monophyletic based on ITS, indicating that more work is needed to clarify the identity of this taxon (FIG. 1).

FIG. 3. Ascomata of *Genea*. a. *G. brunneocarpa* (JC35). b. *G. brunneocarpa* (VK2922). c. *G. compressa* (JC28). d. *G. fageticola* (H139). e. *G. pseudoverrucosa* (GK5286). f. *G. oxygala* (AVM1745). g. *G. tuberculata* (JC32). h. *G. pinicola* (VK2865). i. *Genea* cf. *subbaetica* (VK1342). j. *G. pseudobalsleyi* (CA1). k. *G. vagans* (AH42939). l. *Genea* cf. *anthracina* (GH20100829). m. *G. lobulata* (VK2238). Bars: a 0.5 cm, b 1 cm, c–e 0.5 cm, f–h 1 cm, i–k 0.5 cm, l 0.25 cm, m. 1 cm.

FIG. 4. Ascomata of *Genea*. a. *G. hispidula* (JC23). b. *G. hispidula* (GH20050713). c. *G. hispidula* (NVP). d. *G. arenaria* (RM1140). e. *G. arenaria* (NVH). f. *G. arenaria* (JC8). g. *G. thaxteri* (JC24). h. *G. thaxteri* (JC26). i. *G. thaxteri* (JC27). j. *G. thaxteri* (RM2231). k. *G. thaxteri* (CA4). l. *G. fragrans* (JC21). m. *G. fragrans* (GK4401). Bars: a, b 0.25 cm, c 0.5 cm, d, e 0.25 cm, f 0.5 cm, g 0.25 cm, h–k 0.5 cm, l, m 0.25 cm.

FIG. 5. a–k. Ascomata of *Genea*: a. *G. sphaerica* (GH20100613). b. *G. lespiaultii* (NVJ). c. *G. verrucosa* (AH42941). d. *G. verrucosa* (AH42936). e. *G. verrucosa* (PSS3704). f. *G. verrucosa* (JC1). g. *G. verrucosa* (RM2117). h. *G. verrucosa* (RM2131). i. *G. verrucosa* (CA6). j. *G. verrucosa* (PSS3701). k. *G. verrucosa* (NVM). l–m. Spores of *G. verrucosa*: l. *G. verrucosa* spores (from Vittadini 1831), m.

Combined 28S rDNA - *TEF*1 (FIG. 2) analysis supported the existence of several infrageneric lineages (A–F). Most of these also were supported by ITS (FIG. 1), although ITS data split Genea-B clade into Genea-B1 (*G. fageticola*, *G. oxygala*, *G. tuberculata*) and Genea-B2 (*G. brunneocarpa*, *G. dentata*, *G. pinicola*). Similarly ITS resolved Genea-C into Genea-C1 (*G. lobulata*) and Genea-C2 (*G. gardneri* and *Genea* cf. *subbaetica*). We suspect that *G. bihymeniata* falls in into Genea-C1 with *G. lobulata*, but no ITS data are available. The American species *G. cazaresii* represented the most basal lineage of *Genea* in both ITS and combined 28S-*TEF*1 analyses, which we refer to as Genea-G. No evident apomorphic phenotypical trait could be identified in any of these clades, except for the occurrence of peridia hairs in Genea-F lineage, although it appears that these are sometimes absent in *G. thaxteri*.

The BEAST analysis to estimate divergence times yielded sufficient effective sample sizes (>200) for all relevant parameters, indicating adequate sampling of the posterior distribution. The MCC tree (FIG. 7) was topologically congruent with the inferred combined 28S rDNA - *TEF*1 tree (FIG. 2). Estimates of divergence times and the chronogram are illustrated (FIG. 7). The *Genea* lineage may have split from *Genabea* and *Gilkeya* around 145.5 Mya (95% HPD: 86.7–206.8 Mya). Similar to previous phylogenies based on ITS (Erős-Honti et al. 2008, Guevara-Guerrero et al. 2012), the 28S rDNA data suggest that *G. cazaresii* forms a lineage with *Humaria* and is distantly related to the remaining *Genea* species. The split between *Genea* and the *G. cazaresii*-*Humaria* group was estimated at 84.1 Mya (95% HPD: 47.9–124.8 Mya). The first divergence within *Genea* occured during the Cretaceous, about 76.5 Mya (95% HPD: 42.9–112.7 Mya).

DISCUSSION

We examined authentic specimens of the common European *Genea* species and compared them directly with newly collected specimens of these taxa as well as with the original protologs. When incorporated with the results of molecular analyses these examinations of old and new specimens made it possible to identify the most useful diagnostic features within the genus and resulted in the taxonomic key (see below).

The most controversial species concept is that of the type taxon *G. verrucosa*, because of the conflict between Vittadini's original protolog and the observations made on authentic material by all authors after him regarding spore shape (Berkeley and Broome 1846, Tulasne and Tulasne 1851, Mattirolo 1900a, Ceruti 1960, Montecchi and Sarasini 2000). Without a designated type specimen, the original published illustrations (Vittadini 1831, T.II FIG. VII and T.V FIG. I), probably composed of several collections, must be considered a lectotype. In T.V FIG.I a cross section of a *G. verrucosa* hymenium is depicted (I-a–I-d) along with a single, isolated, rounded spore (I-h, Q = 1.04) and an ascus containing eight spores (I-f, av. Q = 1.20). Vittadini's original specimens in TO contained spores (20–)21.5–30(–34) × (17–)19–24(–26) µm, with Q = (1.15–)1.2–1.43(–1.5) (av. Q = 1.29). The newly collected specimens tentatively identified as *G. verrucosa* had differently shaped spores, ranging from almost spherical or subspherical to subellipsoidal or ellipsoidal, in accordance with previous reports. Hence we propose accepting also subspherical spores as typical of this species. In the present work molecular data revealed also the existence of at least four distinct lineages that are refered to as "*G. verrucosa* complex" because of our inability to designate a type lineage or phenotypically discriminate among them. Both blackish and reddish specimens of *G. verrucosa* can be found intermixed within this complex and neither form constitutes a monophyletic lineage. We observed high ITS divergence between these lineages (7.62% variable sites on average between *G. verrucosa* subclades, 1.15% variable sites on average within each subclade), suggesting that these probably represent cryptic species. This phenomenon is similar to that found with *G. harknessii* in California (Smith et al. 2006). It is possible that some of these cryptic species could be associated with some European species names. One example is *G. perlata*, which has spores similar to *G. verrucosa* and has been considered a synonym (Fischer 1897, Hawker 1954, Ceruti 1960) but was differentiated by a highly wrinkled black ascoma. Additional studies are needed to determine which lineage within the *G. verrucosa* complex is the true *G. verrucosa*. Once this is completed an epitype could be designated to stabilize the taxonomic status of *G. verrucosa*.

Samples of *G. sphaerica* from northern Europe with rounded spore ornaments are not closely related to *Genea* cf. *sphaerica* from Mediterranean Europe that have pointed warts on their spores. Studies of Tulasne's authentic specimens of *G. sphaerica* at TO match with the later descriptions of this species from central Europe (Hesse 1891, Vacek 1951, Hawker 1954) and the newly collected specimens from these areas. Spores are on average 28–31 × 21–24 µm excluding ornamentation, which is formed by hemispherical

←

G. verrucosa spores viewed with SEM (JC20). n–o. Ascomata of *G. verrucosa*: n. *G. verrucosa* (AM2452). o. *G. verrucosa* (VK2132). Bars: a, b 0.5 cm, c 1 cm, d 0.5 cm, e, f 1 cm, g, h 0.5 cm, i 1 cm, j, k 0.5 cm, n, o 5 µm.

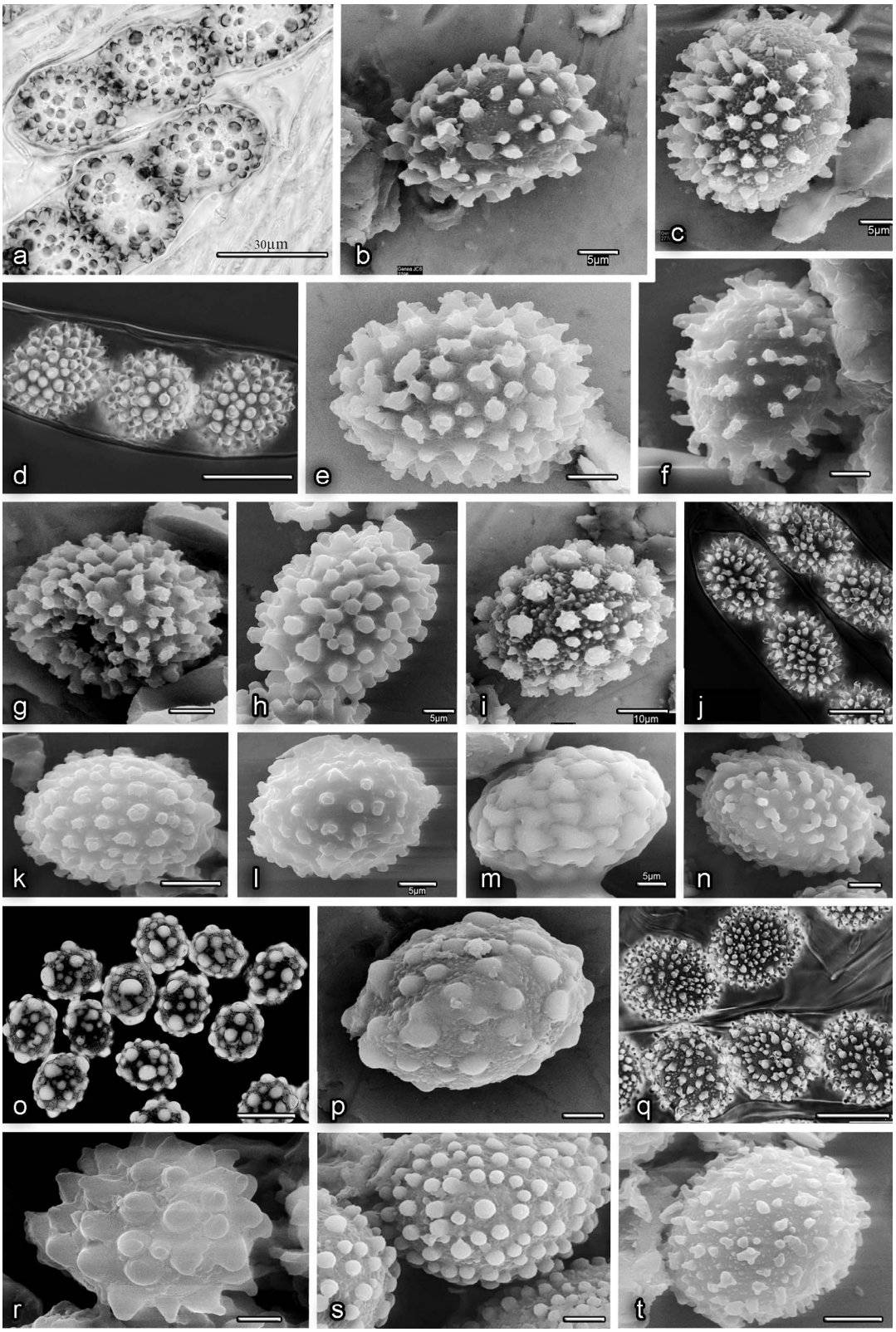

FIG. 6. a. *G. brunneocarpa* spores (JC6). b–c *Genea* spores viewed with SEM. b. *G. brunneocarpa* (JC6). c. *G. dentata* (NVR). d. *G. compressa* spores (JC28). e–i *Genea* spores viewed with SEM. e. *G. compressa* (JC28). f. *G. dentata* (JC19). g. *G. pseudobalsleyi* (CA1). h. *G. fageticola* (GK4129). i. *G. fragrans* (NVQ). j. *G. pinicola* spores (JC12). k–n *Genea* spores viewed with SEM. k. *G. pseudoverrucosa* (GK5088). l. *Genea subbaetica* (BM7). m. *G. lespiaultii* (NVI). n. *G. pinicola* (JC12). o. *G. oxygala* spores (JC14). p–t *Genea* spores

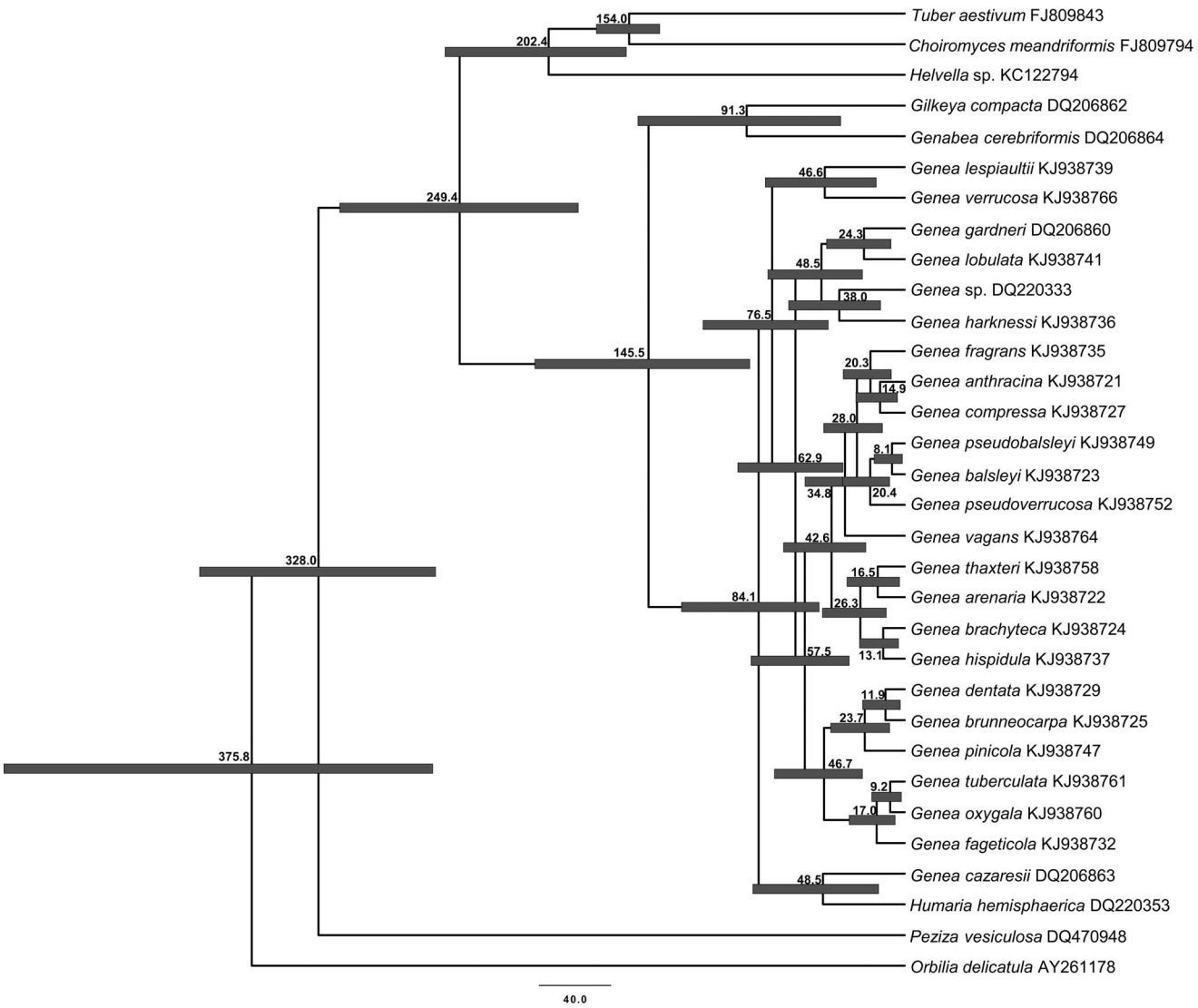

FIG. 7. Divergence time chronogram of *Genea* and related genera. Both mean of estimated divergence time and the 95% highest posterior density bars are indicated at the nodes, based on BEAST analyses.

warts 1–5 µm diam. On the other hand newly collected Mediterranean samples are frequently lobate with spores 24–30 × 20–26 µm excluding ornamentation that consists of spiny or sometimes conical warts 1–2.5 µm high and 1–2 µm wide. These Mediterranean collections fit the description of *G. sphaerica* f. *lobulata* (Moreno-Arroyo et al. 1998a), which was elevated to species rank by Alvarado et al. (2014) as *G. lobulata*. They also may represent the unpublished *G. sphaerica* "f. *insolita*" (Tulasne and Tulasne 1851) and *G. sphaerica* "f. *sporis spinuloso-reticulatis*" (Mattirolo 1903), although authentic material of the latter taxon revealed a different spore ornamentation of slightly larger conic or truncated digitated warts 2–4 µm high × 1–3 µm wide. Spores are also more ellipsoid (24–26.5 × 19–21 µm). These characters are similar to those of *G. verrucosa*. However, because the name published by Mattirolo is invalid, we consider it doubtful. Up to three distinct lineages were identified within the *G. lobulata* complex, but we found no diagnostic features that could be used to distinguish among them, so these are interpreted as putative cryptic

←
viewed with SEM. p. *G. oxygala* (JC16), *Genea* spores q. *G. tuberculata* (JC32). r. *G. vagans* (JC17). s. *G. sphaerica* (NVF). t. *G. lobulata* (BM1043). Bars: a 30 µm, b, c 5 µm, d 30 µm, e–h 5 µm, i 10 µm, j 20 µm, k 10 µm, l–n 5 µm, o 30 µm, p 5 µm, q 25 µm, r–t 5 µm.

species. Spore sizes of *G. lobulata* or *G. sphaerica* do not match those reported for *G. sphaerica* var. *lazzari* (mean 24.5 × 21.5 µm), so the status of *G. sphaerica* var. *lazzari* remains unresolved. Of interest, an ITS rDNA sequence from Oregon (USA) identified as *G. harknessii* (AY927851) is similar to our sequences of *G. lobulata* (FIG. 1), indicating that this group is found on two continents.

Our analyses based on ITS, 28S rDNA and *TEF*1 suggest that *G. sphaerica* is closely related to *G. lespiaultii*, and no significant value supported them as mutually exclusive monophyletic groups. RNA polymerase II second largest subunit (*RPB*2) data also was generated from a single collection from each taxon (primers bRPB2-6F and bRPB2-7R, Matheny 2005), revealing 4/365 differences (GenBank KT950257 and KT950258). We conclude that these genetic markers were insufficient to resolve a putatively recent evolutionary split between these species. In addition, *G. lespiaultii* is one of the most easily recognized species of *Genea* because of its striking flat and irregular spore ornamentation, preventing us from challenging its species status.

Many European samples displayed a significant relationship with *G. arenaria*, from western North America, although they formed distinct lineages (ca. 96% similarity in ITS, 99.6% in 28S rDNA), interpreted as cryptic species. Morphological features of European *Genea* cf. *arenaria* match the holotype (Harkness 1899) and the reexamination of authentic material from Harkness sent by Lloyd to TO herbarium (Smith et al. 2006). This last specimen presents a dull brown verrucose peridium, with tomentum present at deep folds. Spores measure 27–31 × 21–25 µm including ornamentation, which consists in small truncate papillae or cones 1–2 µm high.

A similar situation also can be seen in the clade containing *G. mexicana* (Guevara-Guerrero et al. 2012), *G. thaxteri* from eastern USA and several European collections of *G. verrucosa* f. *badia*, suggesting that these taxa are closely related and perhaps synonyms. Morphologically *G. mexicana* has lobate ascomata with some peridial trichomes while *G. thaxteri* is described as globose-depressed, slightly lobed or without lobes and without peridial hairs (Gilkey 1954). The inner cavity was described as single in *G. thaxteri* but chambered in *G. mexicana*. Spore ornamentation is formed of mostly rounded or conical papillae in *G. thaxteri* whereas *G. mexicana* is reported to have versiform conical, truncate, bi- or trifurcate warts. A comparative study of both types was conducted, revealing spore size is almost identical (24.5–31.0 × 20.0–25.0 in *G. thaxteri* vs. 25.5–31.0 × 18.0–22.0 in *G. mexicana*). Average Q was slightly lower in *G. thaxteri* (Q = 1.27) than *G. mexicana* (Q = 1.42). Comparing these types with other American *G. thaxteri* collections, some degree of variability in spore size among genetically identical specimens was observed, some specimens exceeding the ranges mentioned (up to 36.5 µm). Another variable feature is peridium hairiness. The observation of scarce hairs in *G. thaxteri* type suggests this characteristic is not a reliable diagnostic feature. European specimens of this lineage were variable in color (ranging from light yellow to tan or pale brown), shape (globose to subglobose when young, to lobed or stellate when mature) and in peridium wart morphology, which can include minute papillae to small irregular warts or small polygonal warts with a slightly darkened apex. Spore size varied among specimens, some of them matching the range reported by Gilkey (1939), some others exceeding these values up to 35 µm. Due to these similarities we hypothesize that both taxa represent a single variable species. However, this should be addressed in a specifically focused project where additional collections and markers are studied.

Regarding *G. fragrans* (=*G. klotzschii*), both macro- and microscopical features (black peridium generally without pyramidal warts, large spores ornamented with large truncated warts) can help discriminate this species from *G. verrucosa* (Wallroth 1833, Dietrich and Klotzsch 1839, Berkeley and Broome 1846, Corda 1854, Fischer 1897, Hawker 1954). Of the five authentic specimens of *G. klotzschii* in Berkeley and Broome herbarium in K(M), one is immature (K173360) and two others are in poor condition (K173525, K173526). K173368, from Leigh Woods, Bristol, UK, matches the species concept of *G. sphaerica*. This is probably the specimen mentioned by Hawker (1954) and perhaps the same checked by Tulasne and Tulasne (1851) and Fischer (1897), who reported that spores did not exceed 32 µm. The remaining specimen in Berkeley's and Broome's herbarium is from Stapleton, Bristol (K173527), and has spores 37–40 × 31–25 µm including ornamentation of large truncated warts 3–4 µm high. This is probably the one depicted in Corda (1854) and matches other reports of this species (Fischer 1897, Hawker 1954). The new species *G. fageticola* (Alvarado et al. 2014) can be similar microscopically to *G. fragrans* but has a more globose ascoma without labyrinthic hymenium folds, a peridium usually covered with small polygonal warts instead of minute papillae, epithecium brownish instead of blackish and a large basal tuft of brownish or reddish hyphae. Molecular data indicate that specimens morphologically identified as *G. fragrans* belong to two or three different clades. We could not identify any morphological features to discriminate between these lineages, although specimens were collected in different habitats. Two *G. fragrans* lineages were found with lowland trees such as *Quercus* or *Carpinus*, whereas

the other lineage seems associated with *Fagus sylvatica*. Wallroth (1833) discovered this species under *F. sylvatica* at Straußberg (Thüringen, Germany), but later reports of the same type collection mention an oak forest instead (Dietrich and Klotzsch 1839). Clearly additional studies are needed to resolve the species concepts within this group.

We also report the first molecular data for *G. vagans*, supporting its status as an independent species. Original Italian collections by Mattirolo (1900) have been described and illustrated by Ceruti (1960), and later reports are known from Russia (Bucholtz 1901) and Spain (Vidal 1997). Spore size documented by these authors match our own observations on authentic material from Mattirolo and those made on the new specimens studied in the present work (32–41 × 27–37 µm including ornamentation), although spore ornamentation is slightly smaller (cones 3–6 µm wide × 2–4 µm high) in our recent collections. This species resembles the recently proposed *G. compressa* (Alvarado et al. 2014) but differs because of its spore ornamentation (4–4.5 × 4 µm in *G. compressa*) and the overall spore size (29.5–33 × 25.5–28 µm in *G. compressa*).

Three European samples are related to the North American species *G. anthracina* because their morphology and genetic profile are an excellent match of the isotype (Stewart and Heblack 1979). European specimens are black, have a conspicuous basal tuft of hyphae and are 0.8–1.3 cm diam. The ascomata are covered with polygonal warts that extend through the apical orifice to the internal epithecium, which lines a single, regular inner chamber lacking peridium wall projections. The peridium has scattered long hairs. Asci are 190 × 26 µm on average whereas spores are (23–)25–26(–27) × (17.5–)18–20 (–21)µm (Qm = 1.3) excluding ornamentation, which is formed by small slightly scattered conical-truncate warts, 2.5 × 1.5–2.5 µm (width × height). However, molecular differences between the European specimens and the American isotype prevented us from identifying them all as *G. anthracina* before the potential intraspecific variability of this species is explored with new American collections.

We also found three European collections that are phylogenetically close to the North American taxon *G. gardneri* Gilkey. Of interest, the European collections morphologically fit the description of *G. subbaetica* because of their long asci, small peridium cells, fruiting season (winter but not spring) and the white mycelium that is found in most specimens. In the present work we tentatively used this epithet for these specimens, although the authentic molecular identity of the original concept of *G. subbaetica* could not be obtained from the type or other authentic material (kindly loaned by Dr Baldomero Moreno-Arroyo) after many attempts on different herbarium samples.

Finally, in the present study we attempted to estimate the divergence time of the genus *Genea* and its major lineages. We used a secondary calibration approach according to the closest reliable divergence times estimated by Prieto and Wedin (2013) for Pezizomycetes based on fossils and by Bonito et al. (2013) for the Tuberaceae sister taxa. In spite of the large uncertainty it seems that the genus *Genea* might have diverged in the late Cretaceous at the same time as several infrageneric lineages of *Tuber* (Bonito et al 2013). A more accurate and reliable divergence time estimation could be achieved only through a new analysis involving fossil records or some additional, unlinked loci. In the future a worldwide sampling (especially in Asia and South America) could facilitate the analysis of ancestral area reconstruction of *Genea*.

KEY TO THE GENUS *GENEA*

Prolog: Truffle fungi have not been studied carefully across the globe so the distribution of *Genea* remains incomplete, particularly in Asia where there are likely many undescribed species. Most *Genea* species thus far appear to be restricted to either Europe or North America. Based on these putative distributions we have provided geographical information in the key to make it more useful to future truffle taxonomists (NA = North America, ENA = eastern North America, WNA = western North America, EU - Europe. All measures refer to average values).

1. Ascomata black or reddish black or with polygonal warts, peridium not hairy 2
1. Ascomata yellowish, tan, brownish or reddish, or hairy peridium 18
2. Lacking wall projections, ascomata with a single regular inner cavity, not lobed (ENA) 3
2. With wall projections or with multiple inner cavities, lobed or not 4
3. Peridium composed of a single layer, hemispherical spore ornaments . . . . . *G. anthracina*
3. Peridium composed of two distinct layers, conical spore ornaments . . . . . *G. balsleyi*
4. Wall projections into inner cavity forming large sterile whitish trama plates (EU) 5
4. Wall projections into inner cavity, large whitish trama plates absent 7
5. Spores ornamented with flat warts . . . . . *G. lespiaultii*
5. Spores ornamented with hemispherical or irregular warts 6
6. Spores ornamented with small irregular warts .*G. lobulata*
6. Spores ornamented with hemispherical warts *G. sphaerica*
7. Spores > 32 µm long (ornamentation excluded) 8
7. Spores < 32 µm long (ornamentation excluded) 10
8. Spore covered with low semiglobose papillae (WNA) . . . . . *G. gardneri*
8. Spores ornamented with anvil-shaped warts 9
9. Ascomata lobed, small basal tuft of hyphae . . *G. fragrans*

9. Ascomata globose, abundant basal tuft of hyphae . . . . . *G. fageticola*
10. Paraphyses branched, hymenium interrupted, American (WNA) 11
10. Paraphyses not branched, hymenium continuous, European (EU) 12
11. Spores with small irregular pointed warts < 3 µm high . . . . . *G. harknessii*
11. Spores with rounded warts, hymenial layers sometimes fused . . . . . *G. bihymeniata*
12. Spores with truncated warts 13
12. Spores with irregular, pointed or conical warts 14
13. Spore warts > 2 µm broad . . . . . *G. pseudoverrucosa*
13. Spores warts < 2 µm broad . . . . . *G. pseudobalsleyi*
14. Spores with irregular pointed warts 15
14. Spores with conical warts 17
15. Spore warts > 3 µm high, either fang-like or molar-like . . . . . *G. dentata*
15. Spore warts < 3 µm high 16
16. Spore warts irregular or pointed, fruiting in spring-summer . . . . . *G. verrucosa*
16. Spore warts irregular, pointed or rounded, fruiting in winter . . . . . *G. subbaetica*
17. Spores < 32 µm long, including ornaments 4–4.5 × 4 µm (wide × high) . . . . . *G. compressa*
17. Spores >32 µm long, including ornaments 4–6 × 2–4 µm (wide × high) . . . . . *G. vagans*
18. Ascomata covered with hairs, at least at the apical orifice 19
18. Ascomata lacking hairs 24
19. Spores > 30 µm long 20
19. Spores < 30 µm long 22
20. Spores > 36 µm long, obtuse roundish warts (NA, EU) . . . . . *G. hispidula*
20. Spores sometimes > 30 µm, versiform warts (NA) . . . 21
21. Peridium with scarce hairs . . . . . *G. thaxteri*
21. Peridium hairy . . . . . *G. mexicana*
22. Truncated spore warts narrower than 2 µm (NA) . . . . . *G. arenaria*
22. Conical or rounded warts 2–4(5) µm broad (ENA) . . 23
23. Conical warts, ascus wall thickened throughout . . . . . *G. brachytheca*
23. Rounded warts, ascus wall thickened only at the base . . . . . *G. asperula*
24. Spores < 21 µm long, ornamentation 0.5 µm high (WNA) . . . . . *G. cazaresii*
24. Spores > 21 µm long 25
25. Spores with medium truncated warts 26
25. Spores with large hemispherical or small pointed warts 27
26. Associated with *Pinus* spp. (EU) . . . . . *G. pinicola*
26. Associated with *Quercus* spp. (EU) . . . . . *G. brunneocarpa*
27. Spores with large hemispherical warts (EU) . *G. oxygala*
27. Spores with small pointed warts (EU) . . . *G. tuberculata*

ACKNOWLEDGMENTS

We thank Mr A. Priego and Mr J.A. Pérez of the Electron Microscopy Service of the University of Alcalá de Henares for their help with the SEM. We also thank Luis Monje and Ángel Pueblas of the Department of Drawing and Scientific Photography at the Alcalá University for their help with digital treatment of images; we also thank Dr J. Rejos, curator of AH herbarium, for his assistance with specimens examined in the present study. Financial support for ME Smith was provided by University of Florida's Institute for Food and Agricultural Sciences. We also thank P. Chautrand, A. García Blanco, G. Hensel, R. Martínez, D. Mitchell, A. Montecchi, B. Moreno-Arroyo, G. Pacioni, J.-B. Perez, C.M. Pérez del Amo, P. Ribollet, M.A. Sanz, F. Sáinz, T. Sánchez and J.M. Vidal for kindly sending us their collections and images.

LITERATURE CITED

Altschul SF, Madden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. Nucleic Acids Res 25:3389–3402, doi:10.1093/nar/25.17.3389

Alvarado P, Cabero J, Moreno G, Bratek Z, van Vooren N, Kaounas V, Konstantinidis G, Agnello C, Merényi Z, Smith ME. 2014. Species diversity of *Genea* (Ascomycota, Pezizales) in Europe. Ascomycete.org 6:41–51.

———, Moreno G, Manjón JL. 2012. Comparison between *Tuber gennadii* and *T. oligospermum* lineages reveals the existence of the new species *T. cistophilum* (Tuberaceae, Pezizales). Mycologia 104:894–910, doi: 10.3852/11-254

Berkeley MJ, Broome CE. 1846. Notices of British hypogaeous fungi. Ann Mag Nat Hist 18:73–82.

Bonito G, Smith ME, Nowak M, Healy RA, Guevara G, Cázares E, Kinoshita A, Nouhra E, Domínguez L, Tedersoo L, Murat C, Wang Y, Moreno-Arroyo B, Pfister D, Nara K, Zambonelli A, Trappe J, Vilgalys R. 2013. Historical biogeography and diversification of truffles in the Tuberaceae and their newly identified southern hemisphere sister lineage. PLoS One 8:e52765, doi: 10.1371/journal.pone.0052765

Bucholtz F. 1901. Hypogaeen aus Russland. Hedwigia 40:304–322.

Cázares EJ, García J, Castillo J, Trappe JT. 1992. Hypogeous fungi from northern Mexico. Mycologia 84:341–359.

Ceruti A. 1960. Iconographia mycologica. Suppl. II. In: Elaphomycetales et Tuberales. Trento, Italy.

Corda ACI. Icones Fungorum hucusque cognitorum. . Vol. 6. In: Zobel JB, ed. Prague, Czech Republic: F Ehrlich. p 1837–1854.

Dietrich AG, Klotzsch F. 1839. Flora Regni Borussici: flora des Königreichs Preussen oder Abbildung und Beschreibung der in Preussen wildwachsenden Pflanzen. Berlin: Verlag von Ludwig Ochmigke.

Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian phylogenetics with BEAUti and the BEAST 1.7. Mol Biol Evol 29:1969–1973, doi:10.1093/molbev/mss075

Erős-Honti Z, Kovács GM, Szedlay G, Jakucs E. 2008. Morphological and molecular characterization of *Humaria* and *Genea* ectomycorrhizae from Hungarian deciduous forests. Mycorrhiza 18:133–43, doi:10.1007/s00572-008-0164-7

Fischer E. 1897. Tuberaceen. In: Rabenhorst GL, ed. Kryptogamenflora. 2nd ed. 5. Leipzig, Germany.

Gardes M, Bruns TD. 1993. ITS primers with enhanced specificity for basidiomycetes—application to the identification of mycorrhizae and rusts. Mol Ecol 2:113–118, doi: 10.1111/j.1365-294X.1993.tb00005.x

Gilkey HM. 1939. Tuberales of North America. Corvallis, Oregon: Oregon State College. 63 p.

———. 1954. Tuberales. In: North American Flora. Ser. II, Part 1. p 1–29.

Gori L. 2005. Funghi ipogei della Lucchesia, di altre Province italiane e dall'estero. Maria Pacini Fazzi Ed., Firenze, Italia.

Gross G. 1992. Die Sporenmaße der europäischen *Genea*-Taxa. Z Mykol 58:113–120.

———. 1996. Validierung meiner provisorischen Hypogäen-Taxa. Z Mykol 62:175–180.

Guevara-Guerrero G, Stielow B, Tamm H, Cázares-Gonzales E, Göker M. 2012. *Genea mexicana*, sp. nov., and *Geopora tolucana*, sp. nov., new hypogeous Pyronemataceae from Mexico and the taxonomy of *Geopora* reevaluated. Mycol Prog 11:711–724. doi:10.1007/s11557-011-0781-y

Hansen K, Perry BA, Dranginis AW, Pfister DH. 2013. A phylogeny of the highly diverse cup-fungus family Pyronemataceae (Pezizomycetes, Ascomycota) clarifies relationships and evolution of selected life history traits. Mol Phyl Evol 67:311–335. doi:10.1016/j.ympev.2013.01.014

Harkness HW. 1899. Californian hypogæous fungi. Proc Calif Acad Sci. 3rd ser. Botany 1:241–292

Hawker LE. 1954. British hypogeous fungi. Phil Trans R Soc Lond B 237:429–546. doi:10.1098/rstb.1954.0002

Hesse R. 1894. Die Hypogaeen Deutschlands: II Die Tuberaceen und ElaphomycetenMarburg, Germany.

Higgins D, Thompson J, Gibson T, Thompson JD, Higgins DG, Gibson TJ. 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res 22:4673–4680. doi:10.1093/nar/22.22.4673

Matheny PB. 2005. Improving phylogenetic inference of mushrooms with RPB1 and RPB2 nucleotide sequences (Inocybe; Agaricales). Mol Phyl Evol 35:1–20. doi:10.1016/j.ympev.2004.11.014

Mattirolo O. 1900a. Elenco dei "fungi hypogaei" raccolti nelle Foreste di Vallombrosa negli anni 1899–1900. Malpighia 14:1–24.

———. 1900b. Gli ipogei di Sardegna e di Sicilia. Malpighia 14:39–110.

———. 1903. I fungí ipogei Italiani. Memorie Accad Sci Torino, ser. 2. 53:331–355.

Montecchi A, Sarasini M. 2000. Funghi Ipogei d'Europa. Associazione Micologica Bresadola, Fondazione Centro Studi Micologici Trento, 714 p.

Moreno-Arroyo B, Gómez J, Calonge FD. 1998a. *Genea sphaerica* f.ma *lobulata* f.ma nova dalla Spagna. Boll Grupo Micol G Bresadola 41:205–210.

———, ———, ———. 1998b. *Genea subbaetica* sp. nov., from Spain. Bol Soc Micol Madrid 23:85–89.

Nylander JAA. 2004. MrModeltest 2. Program distributed by the author. Uppsala, Evolutionary Biology Centre, Uppsala University.

Osmundson TW, Robert VA, Schoch CL, Baker LJ, Smith A, Robich G, Mizzan L, Garbelotto MM. 2013. Filling gaps in biodiversity knowledge for macrofungi: contributions and assessment of an herbarium collection DNA barcode sequencing project. PLoS One 8:e62419, doi:10.1371/journal.pone.0062419

Perry BA, Hansen K, Pfister DH. 2007. A phylogenetic overview of the family Pyronemataceae (Ascomycota, Pezizales). Mycol Res 111:549–571, doi:10.1016/j.mycres.2007.03.014

Pfister DH. 1984. *Genea–Jafneadelphus*—a Tuberalean-Pezizalean connection. Mycologia 76:170–172, doi:10.2307/3792850

Posada D. 2011. Collapse: describing haplotypes from sequence alignments 1.2. Available at: http://darwin.uvigo.es/software/collapse.html [Accessed 28 June 2012].

Prieto M, Wedin M. 2013. Dating the diversification of the major lineages of Ascomycota (Fungi). PLoS One 8: e65576, doi: 10.1371/journal.pone.0065576

Rambaut A, Drummond AJ. 2009. Tracer: MCMC trace analysis tool 1.5.0. Available at: http://tree.bio.ed.ac.uk/software/tracer/

Rehner SA, Buckley E. 2005. A *Beauveria* phylogeny inferred from nuclear ITS and EF1-a sequences: evidence for cryptic diversification and links to *Cordyceps* teleomorphs. Mycologia 97:84–98, doi:10.3852/mycologia.97.1.84

Rodriguez F, Oliver J, Marin A, Medina J. 1990. The general stochastic model of nucleotide substitutions. J Theor Biol 142:485–501, doi:10.1016/S0022-5193(05)80104-3

Ronquist F, Huelsenbeck JP. 2003. MrBayes 3: Bayesian phylogenetic inference under mixed models. Bioinformatics 19:1572–1574, doi:10.1093/bioinformatics/btg180

Saccardo PA. 1889. Sylloge fungorum hucusque cognitorum pars. Vol. VIII. Patavii, Italia.

Smith ME. 2007. NATS truffle and truffle-like fungi 15: *Genea balsleyi* sp. nov. (Pyronemataceae), a new hypogeous ascomycete from New Jersey. Mycotaxon 99: 239–244.

———, Trappe JM, Rizzo DM. 2006. *Genea, Genabea* and *Gilkeya* gen. nov: ascomata and ectomycorrhiza formation in a *Quercus* woodland. Mycologia 98:699–716, doi:10.3852/mycologia.98.5.699

Stamatakis A. 2006. RAxML-VI-HPC: maximum likelihood-based phylogenetic analyses with thousands of taxa and mixed models. Bioinformatics 22:2688–2690, doi: 10.1093/bioinformatics/btl446

Stewart EL, Heblack RK. 1979. Hypogeous fungi of Minnesota: *Genea anthracina* sp. nov. Mycotaxon 9:451–458.

Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. 2011. MEGA 5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance and maximum parsimony methods. Mol Biol Evol 28:2731–2739, doi:10.1093/molbev/msr121

Tedersoo L, Suvi T, Larsson E, Kõljalg U. 2006. Diversity and community structure of ectomycorrhizal fungi in a wooded meadow. Mycol Res 110:734–748, doi: 10.1016/j.mycres.2006.04.007

Trappe JM. 1979. The orders, families and genera of hypogeous Ascomycotina (truffles and their relatives). Mycotaxon 9:297–340.

———, Evans F, Trappe J. 2007. Field guide to North American truffles: hunting, identifying, and enjoying the world's most prized fungi. Berkeley, California: Ten Speed Press. 136 p.

———, Molina R, Luoma DL, Cázares E, Pilz D, Smith JE, Castellano MA, Miller SL, Trappe MJ. 2009. Diversity, ecology and conservation of truffle fungi in forests of the Pacific Northwest. Gen. Tech. Rep. PNW-GTR-772. Portland, Oregon: U.S. Department of Agriculture, Forest Service, Pacific Northwest Research Station.

Tulasne L-R, Tulasne C. 1851. Fungi hypogaei. F Klincksieck, ed. Paris. 222 p.

Vacek V. 1951. Zemnička kulovitá—*Genea sphaerica* Tul. Česká Mykol 5:3–5.

Velenovský J. 1922. České Houby 4–5. Prague, Czech Republic: České Botanické Společnosti.

Vidal JM. 1997. Algunos hongos hipogeos nuevos o poco citados de Cataluña (Zygomycotina, Ascomycotina y Basidiomycotina). Rev Cat Micol 20:25–62.

Vilgalys R, Hester M. 1990. Rapid genetic identification and mapping of enzymatically amplified ribosomal DNA from several *Cryptococcus* species. J Bacteriol 172:4238–4246.

Vittadini C. 1831. Monographia Tuberacearum. Milano, Italia. 88 p.

Wallroth KFW. 1833. Flora cryptogamica Germaniae. Vol II. Nuremberg, Germany. 923 p.

White TJ, Bruns T, Lee S, Taylor JW. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ, eds. PCR protocols: a guide to methods and applications. New York: Academic Press Inc. p 315–322.