New records of some Ascomycete truffle fungi from Turkey

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1. Introduction
Most truffle fungi form an ectomycorrhizal association with various trees and shrubs. Many truffle species form this ectomycorrhizal association with specific tree genera. The positioning of Turkey at the convergence of the European flora and the Asian flora predicates a high potential for interesting ectomycorrhizal fungal partners. Little is known about the truffle diversity in Turkey. To date, only 17 Ascomycete truffle species have been reported from Turkey: Choiromyces meandriformis Vittad., Geopora arenicola (Lév.), G. arenosa (Fuckel) S.Ahmad, G. cooperi Harkn., G. sumneriana (Cooke) M.Torre, Picoa juniperi Vittad., P. lefebvrei (Pat.) Maire, Sarcosphaera eximia (Durieu & Lév.) Maire, Terfezia arenaria (Moris) Trappe, T. boudieri Chatin, T. leptoderma Tul., Tirmania pinoyi (Maire) Malençon, Tuber aestivum Vittad., T. borchii Vittad., T. brumale Vittad., T. mesentericum Vittad., and T. nitidum Vittad. (Işıloğlu and Öder, 1995; Afyon, 1996; Oztürk et al., 1997; Solak et al., 1999; Doğan and Öztürk, 2006; Solak et al., 2007; Kaya, 2009; Gücin et al., 2010; Castellano and Türkoglu, 2012; Sesli and Denchev, 2012; Güngör et al., 2013; Türkoglu and Castellano, 2013).

During our recent efforts to identify and catalogue all truffle species from Turkey, we examined all truffle collections known from the country. Here we present 6 Ascomycete truffle taxa identified as new records for Turkey. In addition, we present new localities for the previously reported Choiromyces meandriformis, Picoa juniperi, Terfezia leptoderma, Tuber brumale, Tuber nitidum, and Tuber mesentericum.

2. Materials and methods
Field work was restricted to the Muğla, Antalya, Burdur, Osmaniye, Denizli, Uşak, Samsun, Kastamonu, Bolu, Nevşehir, Konya, and Elazığ provinces in Turkey. These provinces are in the Aegean, the Mediterranean, the Black Sea, the Central Anatolia, and the East Anatolia regions. Some of the specimens were found with the help of a truffle dog, but most truffle specimens were discovered by raking in appropriate habitats. Macromorphological characteristics (size, fresh colour, bruising reactions, and odour) of specimens were recorded, after which each was photographed. Micromorphological characters were recorded from tissue sections rehydrated in water, 3% KOH, or Melzer’s reagent. Spores and sterile tissues were photographed using a light microscope. Each collection was split and a representative specimen was deposited in the herbaria of Muğla Sıtkı Koçman University and the herbarium of Oregon State University.

3. Results
In our study, 12 taxa belonging to 4 families were identified. Brief descriptions of new records are presented along with geographic and phenological information. Author names are given according to Kirk et al. (2008) and fungal names according to Index Fungorum and MycoBank.

3.1. Helvellaceae Fries

3.1.1. Picoa juniperi Vittad
Ascocarp 1–3 cm broad, globose to subglobose; surface blackish or blackish brown, covered regularly with obtuse


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or flat warts (Figure 1a). Gleba off-white to pale yellow, marbled with irregular, pale yellowish veins. Peridium 350–450 µm thick, off-white to yellowish brown, of angular cells 25–50 × 20–25 µm with walls 2 µm thick, surface cells reddish yellow, pigmented cells with walls up to 7 µm thick, sometimes with emergent, septate hairs 25–100 × 3.5–6.5 µm (Figure 1b). Gleba of hyaline, parallel hyphae 4.5–5.5 µm broad with walls ±1 µm thick. Asci randomly dispersed in the gleba, 120 × 80 µm, subglobose with a stem of up to 100 µm long, the walls ±1 µm thick, 8-spored (Figure 1c). Ascospores 20–27 × 20–22 µm, mean = 24.3 × 21.6 µm, with walls ±1 µm thick, broadly ellipsoid, hyaline and smooth at first, pale yellowish to pale green and minutely verrucose when mature (Figure 1d).


3.2. Pyronemataceae Corda

3.2.1. Genea verrucosa Vittad.
Syn.: Genea papillosa Berk.
Ascocarp 5–10 mm broad, very irregular in form; surface dark brown to black, with pyramidal warts (Figure 2a). Gleba with a single, much folded, convoluted, global chamber opening to the outside, and with irregular short ridges intruding into the chamber, lined with black warts and glabrous epithecium similar to the ascocarp surface. Peridium 200–250 µm thick with 3 layers: outer layer 40–90 µm thick, of dark reddish brown, angular cells, 35–90 × 20–30 µm, with walls 6–11 µm thick; middle layer 110–180 µm thick, hyaline, of hyaline, angular cells, 15–30 × 5–20 µm, with walls 2 µm thick; inner layer 40–70 µm thick, of hyaline, interwoven hyphae, 4.5–6.5 µm broad, with walls 2 µm thick; epithecium structure similar to that of the peridium (Figure 2b). Asci in a hymenial palisade embedded under the epithecium, (170–)190–225 × 26–30 µm, cylindrical, broadly rounded at the apex, abruptly narrowed at the base as a short stalk, with walls 2 µm thick, 8-spored (Figure 2c). Ascospores 21–26(–28) × 19–22 µm, mean = 23.3 × 20 µm, excluding ornamentation, broadly ellipsoid, hyaline at first, pale yellow when mature, ornamented with irregular, conical warts, 2 µm tall × 1.5–4.5 µm wide (Figure 2c). Paraphyses hyaline, cylindrical, guttulate, 2–7 µm broad, with walls ±0.5 µm thick (Figure 2d).

Figure 1. Macroscopic and microscopic appearance of Picoa juniperi: a- ascocarp, b- peridium, c- ascus, d- ascospore. Scale bars: c and d =10 µm.
3.2.2. *Genea klotzschii* Berk. & Broome

*Syn.: Genea verrucosa* Klotzsch

Ascocarp 0.4–0.8 mm broad, globose to subglobose, surface blackish brown, with pyramidal warts (Figure 3a). Gleba with a simple, infolded chamber opening to the outside, lined with a black epithecium similar to the ascocarp surface. Peridium 220–250 µm thick with 3 layers: outer layer 40–60 µm thick, dark reddish brown, of angular cells, 30–70 × 20–30 µm, with walls of up to 9 µm thick; middle layer 90–120 µm thick, of hyaline, angular cells, 20–30 × 5–20 µm, with walls 2 µm thick; inner layer 90–100 µm thick, of hyaline, interwoven hyphae, 2.5–9 µm broad, with walls ±0.5 µm thick, with scattered inflated cells, 10–20 × 5–10 µm (Figure 3b); epithecium structure similar to that of the peridium. Asci in a hymenial palisade embedded under the epithecium, (180–)210–260 × 22–25 µm, hyaline, cylindrical, broadly rounded at the apex, abruptly narrowed at the base as a short stalk, with walls 2 µm thick, 8-spored (Figure 3c). Ascospores 24–26(–30) × (15–)18–22 µm, mean = 25.7 × 18.7 µm, excluding ornamentation, ellipsoid, hyaline at first, pale yellow when mature, ornamented with irregular, generally flask-shaped or sometimes forked or cone-shaped warts, 3.5–4.5 × 2.5–3.5 µm, with many scattered smaller, irregular warts 1–2 µm broad (Figure 3c). Paraphyses hyaline, cylindrical, guttulate, 2–7 µm broad, with walls ±0.5 µm thick (Figure 3d).

Specimen examined: Samsun: Çarşamba, under *Corylus* sp., 24 November 2010, Türkoğlu AT-1406.

3.2.3. *Stephensia bombycina* (Vittad.) Tul. & C.Tul.

*Syn.: Genea bombycina* Vittad.

Ascocarp 8–10 cm broad, subglobose to irregular with a basal opening, surface pale brown to reddish brown (Figure 4a). Gleba off-white at first, with yellowish veins when mature. Peridium 750–900 µm thick with 2 layers: outer layer 260–350 µm thick, of hyaline to pale brown, angular, sometimes elongated cells, 20–40 × 10–20 µm, with walls 2 µm thick, but the outermost hyphae darker reddish brown, 8–9 µm broad, with walls 2 µm thick, sometimes with emergent, branched, and septate hairs; inner layer 300–400 µm thick, of hyaline, interwoven to parallel hyphae, 8.5–10.5 µm broad, with walls ±1 µm thick (Figure 4b). Gleba of hyaline, interwoven hyphae 6–7 µm broad with walls ±1 µm thick. Asci in a hymenial palisade in the glebal veins, 180–240 × 22–26 µm, cylindrical-clavate, rounded at the apex, narrowed at the base, with walls ±2 µm thick, 8-spored (Figure 4c). Ascospores 22–26 × 21–26 µm, mean = 24.3 × 23.7 µm, smooth, globose, hyaline, slightly yellowish contents, walls ±1 µm thick (Figure 4d).

Specimen examined: Samsun: Çarşamba, under *Corylus* sp., 24 November 2010, Türkoğlu AT-1404.
3.3. **Pezizaceae** Dumort.

3.3.1. **Terfezia leptoderma** Tulasne & C.Tulasne

Ascocarps 2–4 cm broad, globose to subglobose; surface off-white then rose-lilac, yellowish and finally yellowish brown (Figure 5a). Gleba greyish at first, becoming rosy and bruising lilac when exposed to air, grey-green to olive-green with age, with very broad, off-white sterile veins. Peridium 220–600 µm thick, separable, greyish or
olive-green to pale yellow with a narrow brown zone at the surface; of angular cells, sometimes with inflated cells, 35–90 × 20–45 µm broad, with walls up to ±4 µm thick (Figure 5b). Gleba of hyaline, interwoven to parallel hyphae, 9–11 µm broad, with walls ±1 µm thick. Asci randomly dispersed in the gleba, 70–90 × 65–80 µm, subglobose to ellipsoid, with walls ±2 µm thick, 8-spored (Figure 5c). Ascospores 17–21 × 17–21 µm, mean = 18.9 × 18.7 µm, excluding ornamentation, globose, ornamentation of subcylindrical, truncate to round-tipped spines, 1.8–2.6 µm thick at the base and 3.5–4.5 µm tall, yellowish brown but spore wall darker brown than spines (Figure 5d).


3.3.2. *Terfezia olbiensis* Tulasne & C.Tulasne

Ascocarps 2–4 cm broad, globose to subglobose; surface off-white to pale rose at first, later reddish brown and much wrinkled when mature (Figure 6a). Gleba grey-rose at first, later grey-green to olive-green with sterile veins. Peridium 420–600 µm thick, separable, greyish or olive-green to pale yellow with a narrow, brown zone at the surface; of angular cells, sometimes with inflated cells 40–130 × 25–60 µm broad, with walls ±1 µm thick (Figure 6b). Gleba of hyaline, interwoven to parallel hyphae, 9–11 µm broad, with walls ±1 µm thick. Asci randomly dispersed in the gleba, 60–90 × 60–80 µm, subglobose to ellipsoid, with walls ±2 µm thick, 8-spored (Figure 6c). Ascospores (Figure 6c) 15–19 × 15–18 µm, mean = 17.3–16.8 µm, excluding ornamentation, globose, ornamentation with narrow to broad, truncate cones, 2.5–3.5 µm thick at base and 2.6–4.3 µm tall, yellowish brown (Figure 6d).


3.4. *Tuberaceae* Dumort.

3.4.1. *Choiromyces meandriformis* Vittad.

Ascocarp 2–8 cm broad, subglobose to irregular, sometimes lobed; surface pale yellow to yellowish brown, much wrinkled when dried (Figure 7a). Gleba off-white to pale yellow, marbled with irregular, greyish to yellowish veins. Peridium 500–600 µm thick with 2 layers: outer layer 50–100 µm thick, yellowish brown to reddish yellowish brown, of parallel hyphae, 4–7 µm broad, with walls 1 µm thick; inner layer 500–550 µm thick, pale yellowish to brownish, of angular cells, sometimes inflated to 30–70 × 20–30 µm, with walls 2 µm thick, with scattered parallel hyphae, 9.5–13 µm broad, with walls 2 µm thick (Figure 7b). Gleba of hyaline, interwoven hyphae, 9–13 µm broad, with walls ±2 µm thick. Asci 70–120 × 45–80 µm, clavate to saccate, with walls ±2 µm thick, 8-spored. (Figure 7c). Ascospores 15–18 × 15–18 µm, mean = 14.6 × 14.6

![Figure 5](image_url)
µm, excluding ornamentation, globose, pale yellowish to yellowish brown, ornamentation of cylindrical rods, with indented tips, ±2 µm thick at the base and 3.5–4.5 µm tall (Figure 7d).

Specimens examined: İzmir (Solak et al., 1999); Uşak: Eşme, 18 April 2009, Türkoğlu AT-1087; Bolu: 9 September 2012, Türkoğlu AT-1438; Samsun: Çarşamba, 13 November 2012, Türkoğlu AT-1613.

3.4.2. Tuber brumale Vittad.
Ascocarps 3–5 cm broad, subglobose or irregular, often with slight depressions, surface blackish brown to black with angular, pyramidal to irregularly polygonal or somewhat hexagonal warts, 1–2 mm broad that are depressed at the centre and usually with tiny grooves radiating from the centre (Figure 8a). Gleba initially off-white to grey, later bluish grey, then blackish brown when mature, marbled with off-white veins. Peridium 220–330 µm thick, with 3 layers: outer layer 20–60 µm thick, reddish yellow to deep reddish brown, of hyaline, inflated, irregular cells, 20–35 × 10–15 µm, with walls ±2 µm thick; middle layer 90–130 µm thick, off-white to yellowish brown, of hyaline, inflated irregular cells, 10–35 × 5–10 µm, with walls ±2 µm thick; inner layer off-white to yellowish brown, of hyaline, interwoven hyphae, 3.5–4.5 µm broad, with walls ±2 µm thick (Figure 8b). Gleba of hyaline, interwoven hyphae, 4.5–6 µm broad, with walls ±1 µm thick. Asci (60–)90–130 × (35–)50–110 µm, broadly ellipsoid to sometimes subglobose, sessile, with walls ±2 µm thick, 1–4-spored (Figure 8c). Ascospores (19–)22–31(-36) × 15–19(-21) µm, mean = 26.0 × 16.8 µm, excluding ornamentation, in 1-spored asci 26–31 × 15–18 µm, 2-spored 23–27 × 15–21 µm, 3-spored 19–26 × 13–18 µm, 4-spored 24–28 × 16–19 µm, ellipsoid, yellowish-brown, ornamented with cyanophilic, acutely pointed, spines 6.2–7.0 µm tall (Figure 8d).


3.4.3. Tuber excavatum Vittad.
Ascocarp 1–2 cm broad, globose to subglobose or irregular, with a distinct cavity; surface finely papillate to somewhat coarsely warty, pale yellowish brown to yellowish brown at first, reddish brown when mature (Figure 9a). Gleba off-white to pale yellow at first, later yellowish brown to deep yellowish brown, finally reddish brown, marbled with off-white to pale yellow veins originating from the base of the cavity and branching towards the ascocarp surface. Peridium 260–320 µm thick, off-white to pale yellow with 2 layers: outer layer of inflated, irregular cells, 10–15 ×
5–10 µm, with walls ±2 µm thick; inner layer of hyaline, interwoven hyphae, 2.5–3.5 µm broad, with walls ±2 µm thick (Figure 9b). Gleba of hyaline, interwoven hyphae, 3.5–5 µm broad, with walls ±1 µm thick. Asci 60–100 × 40–75 µm, subglobose to ellipsoid, short-stalked, with walls 2–4 µm thick, 1–4-spored (Figure 9c). Ascospores
**3.4.4. Tuber nitidum** Vittad.

Ascocarp 1–3 cm broad, globose to subglobose, the surface generally glabrous even at maturity, somewhat coarsely warty, off-white to pale yellow, rarely with reddish yellow patches (Figure 10a). Gleba white at first, later off-white to pale yellow, marbled with distinct, broad white veins. Peridium 250–550 µm thick with 2 layers: outer layer 50–60 µm thick, pale yellow, of isodiametric to irregular, pigmented cells, 10–20 × 4–10 µm, with walls ±2 µm thick; inner layer 200–500 µm thick, off-white to grey, of hyaline, interwoven to parallel hyphae, 3.5–5.5 µm broad, with walls ±2 µm thick (Figure 10b). Gleba of hyaline, parallel hyphae, 5.5–7 µm broad, with some inflated cells, 10–20 × 5–10 µm, with walls ±1 µm thick. Asci 70–100 × 40–60 µm excluding stalk, broadly clavate, stalk up to 26 µm long, the walls ±2 µm thick, 1–4-spored (Figure 10c). Ascospores 21–33(−39) × (13–)16–27(−29) µm, mean = 28.5 × 21. µm, excluding ornamentation, in 1-spored asci 30–39 × 26–27 µm, 2-spored 26(−31)–32 × 18–22(−29) µm, 3-spored 21–27 × 18–22 µm, 4-spored 22–26 × (13–)16–18 µm, ellipsoid, yellowish brown to reddish brown, ornamented with pointed spines 3.5–4.5 µm tall (Figure 10d).


**3.4.5. Tuber rufum** Pico

Syn.: Oogaster rufus (Pico) Corda

Ascocarp 1–3 cm broad, subglobose to irregular, the surface finely papillate to somewhat warty, yellowish brown to reddish brown, sometimes mottled nearly black (Figure 11a). Gleba white at first, becoming pale reddish-brown to deep reddish brown, marbled with distinct, broad, reddish brown to off-white veins. Peridium 350–480 µm thick with 2 layers: outer layer pale yellow to yellowish brown, of hyaline, isodiametric or irregular, pigmented cells, 10–15 × 7–10 µm, with walls ±2 µm thick; inner layer off-white, of hyaline, mostly interwoven, somewhat parallel hyphae,
3.5–4.5 µm broad, with walls ±2 µm thick (Figure 11b). Gleba of hyaline, interwoven to parallel hyphae, 3.5–7.0 µm broad, with walls ±1 µm thick. Asci 50–90 × 40–50 µm excluding stalk, clavate, stalk of up to 30 µm long, walls 2–3.5 µm thick, 1–5-spored (Figure 11c). Ascospores (22–)26–33(–36) × 17.5–22 µm, mean = 27 × 19 µm, excluding ornamentation, in 1-spored asci 20–37 × 17–20 µm, 2-spored 26–32 × 18–22 µm, 3-spored 23–33 × 17–18 µm, ellipsoid, yellowish brown, ornamented with pointed spines, 3.5–5.4 µm tall (Figure 11d).


3.4.6. *Tuber mesentericum* Vittad.

Ascocarps 2–4 cm broad, subglobose or irregular, with a cavity; surface blackish brown to black with a acutely pyramidal warts, warts irregularly polygonal, 4–6-sided, 2–4 mm broad, usually with tiny grooves radiating from the centre (Figure 12a). Gleba initially off-white, later brown, marbled with off-white and brown veins. Peridium 220–550 µm thick, with 3 layers: outer layer 50–150 µm thick, blackish brown, of hyaline, inflated, angular cells, 10–20 × 5–10 µm, with walls ±2 µm thick; inner layer 150–400 µm thick, off-white, of hyaline, interwoven hyphae, 3.5–6 µm broad, with walls ±2 µm thick (Figure 12b). Gleba of hyaline, interwoven hyphae, 3.5–7 µm broad, with walls ±1 µm thick. Asci 55–75 × 40–60 µm, saccate to shortly pedicellate, with walls ±2 µm thick, 1–4-spored (Figure 12c). Ascospores (22–)26–35(–38) × 21–30(–36) µm, mean = 29.6 × 26.2 µm, excluding ornamentation (Figure 12c), in 1-spored asci (27–)30–33(–38) × (23–)27–36 µm, 2-spored 26–35 × 23–28(–36) µm, 3-spored (26–)28–33 × (23–)26–30 µm, 4-spored 22–29 × 21–25 µm, rarely globose but generally ellipsoid to broadly ellipsoid, yellowish-brown, ornamented with a very reticulate-alveolate, up to 5.5 µm tall, 3–5 meshes across the spore (Figure 12d).

Specimen examined: Denizli (Castellano and Türkoğlu, 2012); Denizli: Bozkurt, 15 June 2012 Türkoğlu AT-1437.

4. Discussion

We report 6 Ascomycete truffle taxa found in Turkey for the first time: *Genea klotzschii*, *Genea verrucosa*, *Stephensia bombycina*, *Terfezia olbiensis*, *Tuber excavatum*, and *Tuber rufum*. We also report new localities within Turkey for *Choiromyces meandriformis*, *Picoa juniperi*, *Terfezia leptoderma*, *Tuber brumale*, *Tuber nitidum*, and *Tuber mesentericum*.

We report the genera *Genea* and *Stephensia* from Turkey for the first time. *Genea klotzschii* has fairly long...
asci and spores ornamented with irregular, flask-shaped or fork-shaped warts while *Genea verrucosa* has mostly shorter asci and spores ornamented with irregularly conical warts. *G. verrucosa* occurs in calcareous soils under *Quercus pubescens* in the Mediterranean region while *Genea klotzschii* occurs in acidic soils under *Corylus*.
sp., in the Black Sea region. *Stephensia bombycina* has characteristic smooth, globose spores. Montecchi and Sarasini (2000) reported *S. bombycina* from under *Tilia* but our collections were from acidic soils under *Corylus* sp., in the Black Sea region. *Terfezia olbiensis* has small ascocarps with pale colours and also fairly short spores ornamented with narrow to broadly truncate cones. It appears to be the most uncommon of the *Terfezia* species found in Turkey. Montecchi and Sarasini (2000) reported *T. olbiensis* from under *Quercus* or *Pinus* spp., but our collections occurred in sandy soils associated with *Helianthemum* spp. The combination of a fine to coarsely warted peridium and distinct basal cavity in *Tuber excavatum* easily separates this species from all others in the genus. *T. excavatum* is collected in calcareous soils under mixed *Quercus* spp. and *Pinus* spp. It appears to be closely associated with coniferous trees throughout Europe as well as in Turkey. The spiny spores of *Tuber rufum* are distinctive among the palely coloured *Tuber* species. *T. rufum* occurs in calcareous soils under mixed *Quercus* spp. and *Pinus* spp. It appears to be closely associated with coniferous trees. *G. klotzschii*, *G. verrucosa*, and *S. bombycina* are not desirable edible fungi due to texture or small size. *T. olbiensis*, *T. excavatum*, and *T. rufum* are edible and have economic value, but only *T. olbiensis* is well known, eaten, and sold in local village bazaars.

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