Phylogenetic and morphological studies in *Otidea alutacea* and *O. bufonia* clades (*Pezizales*), with the new species *Otidea adorniae*

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Abstract: *Otidea adorniae* is here proposed and described as new to science upon collections made in xerophilous broadleaved forests of Apulia (southern Italy). Color images and hand drawings illustrating its main diagnostic features are provided. Its position in the complex of *Otidea alutacea* is discussed on the basis of morphological descriptions and phylogenetic analysis of ITS and 28S rDNA data. The new combination *Otidea parvispora* is also proposed. The holotypes of *Otidea cinerascens* and *O. kunmingensis* are examined respectively both morphologically and phylogenetically. In addition, an updated phylogenetic study of the *Otidea bufonia* clade suggests that *Otidea pruinosa* should be regarded a synonym of *Otidea subpurpurea* (= *O. bicolor*).
Keywords: Ascomycota, *Pyronemataceae*, Italy, ribosomal DNA, taxonomy.

Riassunto: *Otidea adorniae* viene qui proposta e descritta come specie nuova su raccolte effettuate nei boschi xerofili di latifoglie della Puglia. Vengono fornite foto a colori e disegni al tratto che ne illustrano i caratteri primari. Viene discussa la sua posizione all'interno del complesso di *Otidea alutacea* su basi morfologiche e attraverso l'analisi filogenetica del ITS e 28S rDNA. Viene anche proposta la nuova combinazione *Otidea parvispora*. Gli holotypi di *Otidea cinerascens* e *O. kunmingensis* sono stati esaminati rispettivamente dal punto di vista morfologico e filogenetico. In aggiunta uno studio filogenetico aggiornato e condotto sul clado di *Otidea bufonia* suggerisce che *Otidea pruinosa* debba essere considerata sinonimo di *Otidea subpurpurea* (= *O. bicolor*).
Parole chiave: Ascomycota, *Pyronemataceae*, Italia, DNA ribosomiale, tassonomia.

Introduction

Liu & Zhuang (2006) were the first to suggest that samples identified as *Otidea alutacea* (Pers.) Massee could actually represent a complex of closely related species. This view was supported by Hansen & Olariaga (2015) and Olariaga *et al.* (2015). The /alutacea clade is a difficult group not only because of the scarce morphological differences observed among the specimens involved, but also due to many taxonomical and nomenclatural problems. In fact, it is thought that some old species could belong in this complex (Olariaga *et al.*, 2015; Xu *et al.*, 2018). However, the only taxon genetically confirmed as member of this clade is *Otidea alutacea* var. *parvispora* Parslow & Spooner, a variety with a less intense yellow pigmentation, smaller ascospores and shorter asci (Parslow & Spooner, 2015).

In 2008 we collected a specimen of *Otidea* macroscopically resembling a typical *O. alutacea*, but with much smaller ascospores. It was originally found only in two localities, Bosco dei Lucci and Bosco Colemi, in Brindisi province (Apulia, Italy), but additional samples from different localities of the same region have been found since then. This small-spored taxon was analyzed genetically, and labeled in Hansen & Olariaga (2015) and Olariaga *et al.* (2015) as "*O. alutacea* (9) IT" and "*O. alutacea* (17) IT", respectively, because it did not match any other known genetic lineage in the /alutacea clade. Parslow & Spooner (2015) published a phylogenetic tree where a sequence from this lineage (KM010069) clusters with two British collections (KT818926, EU784381). Recently, Xu *et al.* (2018) accommodated it into their "clade 5".

On the other hand, some species have been characterized in the group of *Otidea bufonia* with the aid of genetic data (Carbone *et al.*, 2017; Hyde *et al.*, 2017; Xu *et al.*, 2018), but the phylogenetic relationships of these species with the older taxa have not been studied in depth.

The main goals of the present study are: i) investigate the Italian small-spored collections evaluating their position in the /alutacea clade and looking for existing names that match them; ii) re-evaluate the taxonomical status of *Otidea alutacea* var. *parvispora*; and iii) produce an updated phylogeny of the /bufonia clade to evaluate the status of known species.

Material and methods

Morphological study. — The microscopical studies were based on both fresh and dried specimens. Two optical microscopes were used: Optika B353 and Olympus CX41 trinocular with plan-achromatic objectives 10×, 40×, 60×, 100× oil immersion. The following main reagents were used: Melzer's reagent, cotton blue (lactophenol and acid lactic), Congo red, 5% KOH. Water mounts were used for the observation of the pigmentation and measurements. At least 25–30 ascospores naturally discharged from the asci were measured from each mature collection.

Phylogenetic studies. — Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980) or using Fungal DNA Extraction Kit (Bio Teke Corporation, China). PCR amplification was performed with the primers ITS1F and ITS4 (White *et al.*, 1990; Gardes & Bruns, 1993) for ITS region, while LR0R and LR5 (Vilgalys & Hester, 1990; Cubeta *et al.*, 1991) were used to amplify the 28S rDNA region. PCR reactions were performed under a program consisting of a hot start at 95 °C for 5 min, followed by 35 cycles at 94 °C, 54 °C and 72 °C (45, 30 and 45 s respectively) and a final 72 °C step 10 min. PCR products were checked in 1% agarose gels, and positive reactions were sequenced with one or both PCR primers. Chromatograms were checked and reading errors were corrected.

Two combined alignments were built for the /bufonia and /alutacea clades, including ITS1-5.8S-ITS2 (or ITS2 only) + 28S rDNA data. BLAST (Altschul *et al.*, 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration (INSDC) public databases. Sequences came mainly from Hansen & Olariaga (2015), Olariaga *et al.* (2015), Carbone *et al.* (2017) and Xu *et al.* (2018). New sequences have been deposited in GenBank (Table 1). Sequences first were aligned in MEGA 5.0 (Tamura *et al.*, 2011) software with its Clustal W application and then corrected manually. The final alignment of /alutacea clade included 44 samples with 207/541 (ITS1-5.8S-ITS2 rDNA) and 74/390 (28S rDNA) variable sites, while the alignment of /bufonia clade included 81 samples with 140/345 (5.8S-ITS2 rDNA) and 216/832 (28S rDNA) variable sites. Each alignment was loaded in MrBayes 3.1 (Ronquist & Huelsenbeck, 2003) where a Bayesian analysis was run (data partitioned, GTR+G model, two simultaneous runs, six chains, temperature set to 0.2, sampling every 100th generation) until standard deviation fell below 0.01 after 7.8M (/alutacea) or 3.38M

Table 1 – Sequences newly generated for this study, and GenBank accession numbers.

Taxon	Voucher	GenBank Accession Numbers	
		ITS	28S nrDNA
Otidea adorniae	MCVE 30104	MK850482	MK850498
Otidea adorniae	MCVE 30105	MK850483	MK850499
Otidea adorniae	MCVE 30106	MK850484	MK850500
Otidea adorniae	MCVE 30103	MK850485	MK850501
Otidea adorniae	MCVE 30102	MK850486	MK850502
Otidea flavidobrunneola	GDOR 4358	MK850487	—
Otidea flavidobrunneola	TUR-A 183241	MK850488	—
Otidea kunmingensis	HKAS 49452	MK850489	—
Otidea parvispora	MCVE 30107	MK850490	MK850503
Otidea parvispora	MCVE 30108	MK850491	MK850504
Otidea bufonia	TUR-A 148640	MK850492	MK850505
Otidea bufonia	TUR-A 208338	MK850493	MK850506
Otidea bufonia	TUR-A 208339	MK850494	MK850507
Otidea bufonia	TUR-A 208340	MK850495	MK850508
Otidea bufonia	TUR-A 208341	MK850496	MK850509
Otidea bufonia	TUR-A 208342	MK850497	MK850510

(/bufonia) generations. Finally, a full search for the best-scoring maximum likelihood (ML) tree was performed in RAxML (Stamatakis, 2006) using the standard search algorithm (data partitioned, GTR-MIX model, 2000 bootstrap replications). Significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Studied or sequenced collections

***Otidea adorniae*.** Italy. Puglia, Brindisi, "Bosco dei Lucci", alt. 50 m a.s.l., under *Quercus suber* and *Q. ilex*, 10.I.2008, *leg.* C. Agnello (MCVE 30099). *Ibidem*, 05.XII.2008, *leg*. C. Agnello (MCVE 30092). *Ibidem*, 16.XII.2008, *leg*. C. Agnello (MCVE 30100). *Ibidem*, 19.XII.2008, *leg*. C. Agnello (MCVE 30101). *Ibidem*, 23.XII.2008, *leg*. C. Agnello (MCVE 30102, **holotype**). *Ibidem*, 24.XI.2009, *leg*. C. Agnello (MCVE 30103). *Ibidem*, 11.X.2010, *leg*. C. Agnello & M. Carbone (S-F257085). *Ibidem*, 20.XI.2012, *leg*. C. Agnello (MCVE 30095). *Ibidem*, 07.XII.2013, *leg*. C. Agnello (MCVE 30096). *Ibidem*, 07.XI.2015, *leg*. C. Agnello (MCVE 30097). Tuturano-Brindisi, "Bosco Colemi", alt. 45 m a.s.l, under *Quercus ilex* and *Q. suber*, 05.XI.2009, *leg*. C. Agnello (MCVE 30093). *Ibidem*, 24.X.2010, *leg*. C. Agnello (MCVE 30094). Lecce, Otranto, Loc. Frassanito, under *Quercus ilex*, 08.XI.2018, *leg*. V. Kaounas & M. Carbone (MCVE 30104). Brindisi, Cellino S. Marco, "Bosco Curtipetrizzi", alt. 50 m a.s.l., under *Quercus ilex*, 05.XII.2018, *leg*. C. Agnello (MCVE 30098). Lecce, Vernole, Le Cesine Natural Reserve, under *Quercus ilex*, 08.XI.2018, *leg*. V. Curcio (MCVE 30105). Foggia, Motta Montecorvino, Monte Sambuco, under *Pinus* sp., *Quercus cerris* and *Fraxinus ornus*, 05.XI.2010, *leg*. A. Conte (MCVE 30106). ***Otidea bufonia*.** Finland. Varsinais-Suomi, Lohja, Virkkala, Pähkinäniemi, herb-rich deciduous forest on calcareous, mull soil, with *Corylus avellana*, *Betula pendula*, *Populus tremula*, *Picea abies* and *Pinus sylvestris*, 28.VIII.1997, *leg*. J. Vauras (TUR-A 148640). Italy. Calabria, Cosenza, San Demetrio Corone, Calamia, under *Castanea sativa* and *Quercus frainetto*, 1.XI.2010, *leg*. C. Lavorato (TUR-A 208342). Tuscany, Grosseto, Scarlino, Cala Violina, under *Quercus ilex* and *Q. cerris*, 21.XI.2008, *leg*. M. Carbone (TUR-A 208338). Piemonte, Alessandria, Vignole Borbera, Fraz. Variano, under *Quercus* and *Castanea sativa*, 22.XI.2012, *leg*. M. Carbone (TUR-A 208339). Puglia, Lecce, Melendugno, Masseria Bosco Mazza, under *Quercus ilex*, 1.XII.2008, *leg*. C. Agnello (TUR-A 208340). Emilia-Romagna, Modena, Frassinoro, under *Fagus sylvatica*, 23.X.2010, *leg*. P. Ferrari (TUR-A 208341). ***Otidea cinerascens*.** Czech Republic. Hodonín, Žarošice, VIII.1940, Collectio fungorum J. Velenovský 29/1947 (PRM 151779, **holotype**). ***Otidea flavidobrunneola*.** Finland. Varsinais-Suomi, Turku, Ruissalo, Botanical Garden, West part, margin of deciduous forest with *Quercus robur*, *Corylus avellana* and *Betula* sp., on clayey soil, 4.IX.2009, *leg*. J. Vauras (TUR-A 183241). Italy. Piemonte, Cuneo, Ormea, Loc. Pornassino, 1200 m a.s.l., under *Corylus avellana* and *Fraxinus excelsior*, 21.VII.2018, *leg*. F. Boccardo (GDOR 4358). ***Otidea kunmingensis*.** China. Yunnan, Kunming, alt. 1980 m a.s.l., on the ground under *Quercus*, 8.X.2004, *leg*. Z. L. Yang (HKAS 49452, **holotype**). ***Otidea parvispora*.** Greece. Lesvos, on mossy ground under *Pinus brutia*, *Quercus coccifera*, *Pyrus amygdaliformis* and *Quercus pubescens*, 25.XI.2018, *leg*. G. Fransouas (MCVE 30107). Spain. Basque Country, Bizkaia, Zamudio, Jardin urbano, under *Quercus robur* and *Laurus* sp. 18.XII.2018, *leg*. J. Ruiz (MCVE 30108).

Phylogenetic results

Analysis of ITS-28S rDNA sequences of the /alutacea clade (Fig. 1) produced an overall topology not different from those published before by Hansen & Olariaga (2015), Olariaga *et al*. (2015), and Xu *et al*. (2018). Eleven clades were found to be significantly different, ten of them already identified by previous authors, plus a new one, formed by the holotype of *Otidea kunmingensis*. The sequences obtained from the samples of *Otidea alutacea* var. *parvispora* studied in the present work matched those of *O. alutacea* lineage 1 in Xu *et al.* (2018), while the remaining samples of the /alutacea clade showed significant genetic similarities with *O. alutacea* lineage 5, represented by MC2010-05 (S). These genetically distinct lineages have diagnostic morphological features, so they are treated here as an independent species after the proposal of the necessary taxonomical changes.

The analysis of the /bufonia clade (Fig. 2) supported the identification of some samples as *O. flavidobrunneola*. The remaining specimens were nested in the complex of *O. bufonia*. Two of them (MCVE 29371 and MCVE 29372) probably represent a basal lineage differing from three closely related clades: one contains the types of *O. subpurpurea*, *O. pruinosa* and *O. bicolor*, while the other two are exclusively formed by samples identified as *O. bufonia*, in agreement with the results obtained by Xu *et al.* (2018).

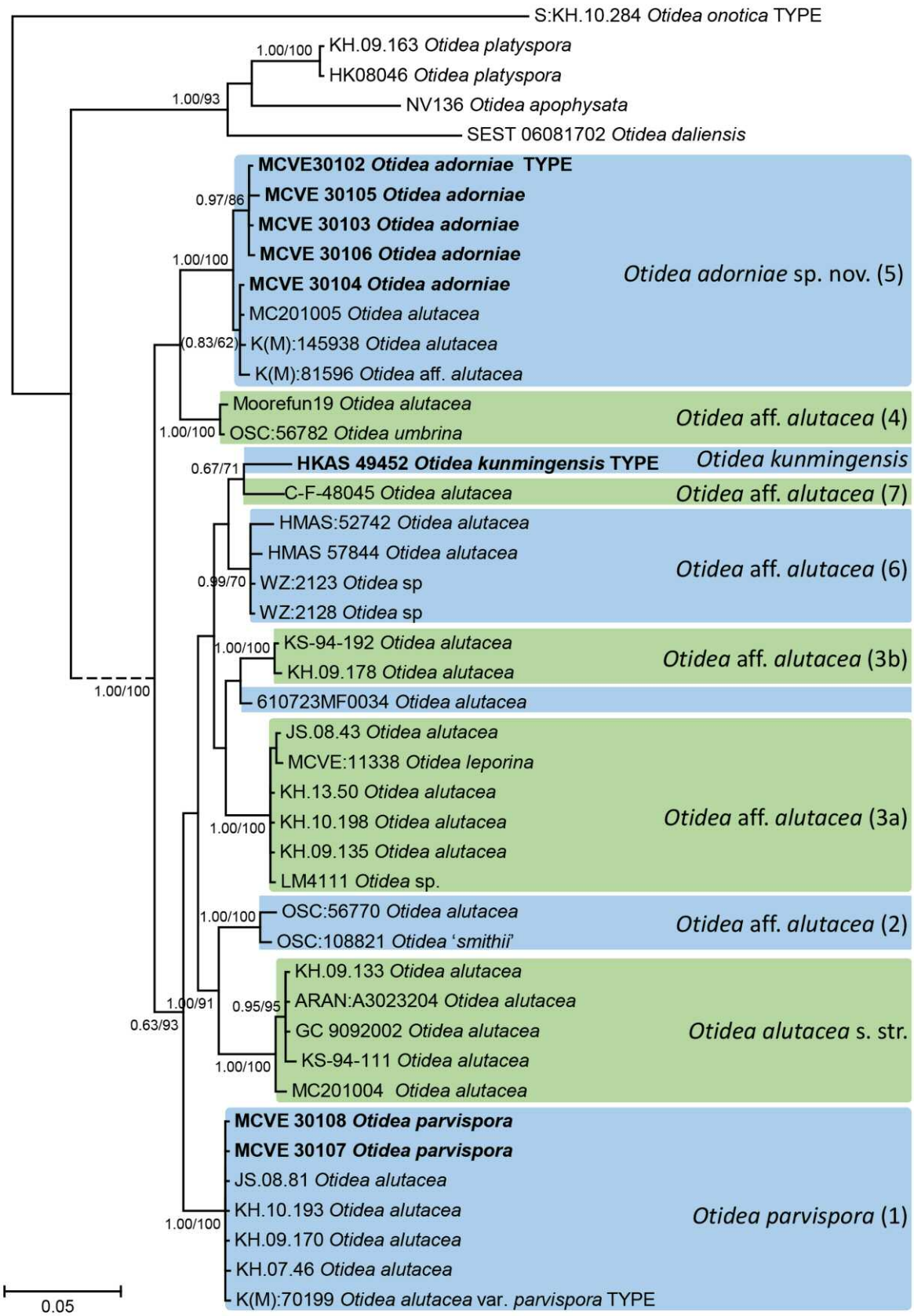

Fig. 1 – Consensus phylogram of the /alutacea clade obtained in MrBayes 3.1 from of a combined alignment of ITS rDNA and 28S rDNA. Dashed branch was shortened for publication. Nodes were annotated if supported by > 70 % ML BP or >0.95 bayesian PP, but nonsignificant support values are exceptionally represented inside parentheses.

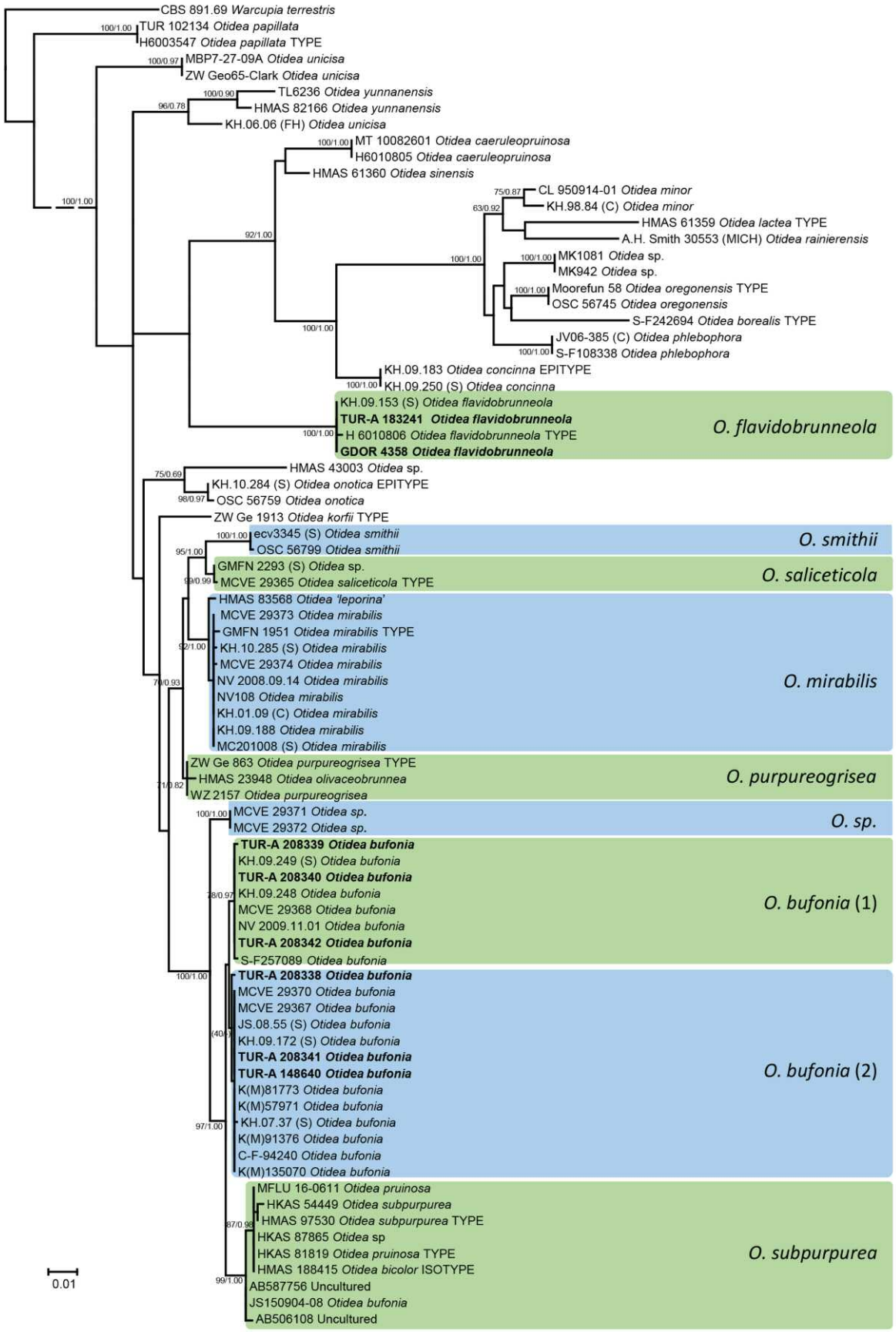

Fig. 2 – Consensus phylogram of the /bufonia clade obtained in MrBayes 3.1 from of a combined alignment of ITS rDNA and 28S rDNA. Dashed branch was shortened for publication. Nodes were annotated if supported by > 70 % ML BP or >0.95 Bayesian PP, but nonsignificant support values are exceptionally represented inside parentheses.

Taxonomy

Otidea parvispora (Parslow & Spooner) M. Carbone, Agnello, Kautmanová, Z.W. Ge & P. Alvarado, *comb. et stat. nov.* – MB 830673

Basionym: *Otidea alutacea* var. *parvispora* Parslow & Spooner, *Ascomycete.org*, 7 (6): 297 (2015).

Otidea adorniae Agnello, M. Carbone & P. Alvarado, *sp. nov.* – MB 830672

Diagnosis: Besides its unique genetic profile, it differs from *Otidea alutacea* by its smaller ascospores, which are also more ellipsoid and less "cylindroid", and from *O. parvispora* because of the slightly shorter and wider ascospores having a slightly lower average Q.

***Holotypus hic designatus*:** Italy. Brindisi, "Bosco dei Lucci", alt. 50 m a.s.l., coordinates: 40° 57' 30" N, 17° 87' 48" E, under *Quercus suber* and *Q. ilex*, 23.XII.2008, *leg*. C. Agnello (MCVE 30102).

Etymology: Dedicated to Mrs. Francesca Adorni for her great love, passion and sacrifices for the protection and conservation of the wood called "Bosco dei Lucci", the locality where the holotype was found.

Macroscopical features

Apothecia gregarious, up to 7 cm in diameter and 5 cm high, ear-shaped when young then more deeply cup-shaped, split on one side and irregularly lobed, subsessile to slightly stipitate in young and isolated samples. **Hymenium** smooth, ochraceous brown to brown, more or less wrinkled in the center. **Receptacle surface** minutely furfuraceous-warty, hygrophanous, wrinkled toward the base, yellowish brown to ochraceous yellow, paler when drying to almost withish in some specimens. **Basal tomentum** and **mycelium** whitish. **Spore print** white.

Microscopic features

Ascospores smooth, hyaline, regularily ellipsoid, sometimes slitghly inequilateral or subcylindroid, with two guttules (living spores), (10.5–) 11–12 (–12.5) × 6–6.5 (–7) µm [X = 11.8 × 6.4 µm], Q = 1.7–1.95 (Qm = 1.85), with wall up to 0,5 µm thick. **Paraphyses** cylindrical, septate, branched at the base, sometimes anastomosed, slightly longer than the asci, 2.5–3.5 µm wide, tips bent enlarged up to 4–4.5 (–5) µm wide, often with a low notch on the underside. **Asci** cylindrical, 140–170 × 8–10 µm, 8-spored, operculate, pleurorhynchous. **Subhymenium** visible as a small and bit darker zone, composed of cylindrical cells, densely arranged. **Medullary excipulum** arranged as a *textura intricata*, composed of hyaline hyphae, 5–8 µm wide and with wall up to 0.5 µm thick. **Ectal excipulum** of *textura globulosa-angularis* in the inner part, with cells slightly thick-walled, hyaline to very pale yellowish, 10–20 (–25) µm in diameter; in the outer part (i.e. the warts) composed by chains of 4–6 more or less elongated cells, constricted at the septa. Resinous exudates mostly absent, dissolving in Melzer's reagent. **Basal mycelium** composed by hyaline hyphae, septate, 3–4 µm wide, wall thin but in some hyphae up to 0.5 µm thick, mostly smooth.

Habitat and phenology

All the Italian collections grew in a typical xerophilous mediterranean forest dominated by evergreen oaks in calcareous soil. The main trees present were *Quercus ilex* and *Q. suber*, although other species were sometimes present (i.e. *Quercus macrolepis*, *Q. pubescens* or *Q. trojana*). According to the collector's notes, the sample found in Motta Montecorvino seems to have a slightly different habitat due to the presence of *Pinus*, *Fraxinus* and *Quercus ceris*.

ITS rDNA sequences stored in GenBank and published by Parslow & Spooner (2015) suggest that *Otidea adorniae* is probably present in the United Kingdom. The British collection K(M)81596 was found under *Quercus* sp., while K(M)145938 was reported to be found "on soil of flower bed".

The fruiting period in Italy seems to be pretty constant, from October to January.

Study of *Otidea cinerascens* holotype

The holotype (PRM 151779) is composed of one apothecium in pretty good condition for its morphological study. The external surface is minutely warty, ochraceous brown to alutaceous; the hymenium is a bit darker.

Microscopic features

Ascospores ellipsoid, oblong, or subcylindroid, slightly inequilateral, with two oil drops, smooth, hyaline, (13.5)14.5–16.5(17) × 6.5–7.5(8) µm, Q=1.7–2.1, Qm= 1.95. **Paraphyses** curved to slightly hooked, of the same width or very slightly enlarged at tips, 3–4 µm wide, without notches. **Asci** 140–160 × 10–12 µm, 8-spored, operculate, pleurorhynchous. **Subhymenium** visible as a moderately darker zone, cells cylindrical, densely arranged. **Medullary excipulum** of *textura intricata*, hyphae thin-walled to slightly thick-walled, hyaline. **Ectal excipulum** of *textura subglobulosa-angularis*, inner cells hyaline to pale yellowish-brown, 10–20 diam. (globose ones) or 10–25 × 10–18 µm (elongated ones); warts composed of fasciculate, hyphoid hairs, made of 5–6 globose to club-shaped cells, constricted at septa, 8–10 µm wide. Resinous exudates absent to scarce.

Discussion

About *Otidea adorniae* and the /alutacea clade

Otidea adorniae is very difficult to discriminate macroscopically from *O. alutacea* and the other species of the /alutacea clade. On the contrary, both are easily separable microscopically due to the much shorter and thinner ascospores of *O. adorniae*. At present, *O. alutacea*, as epitypified by Carbone (2011), has ascospores (14.5)15–17(18) × 6.5–7 (7.5) µm [15.5–17.5(–18.5) × (6.5–)7–8 (–8.5) µm according to Parslow & Spooner (2013)]. *Otidea parvispora* has also small ascospores, but *O. adorniae* has a slightly smaller average Q (1.85 vs 2.04) if compared with data provided by Parslow & Spooner (2015; see also Van Vooren, 2017). The spore measurements obtained from the two collections of *O. parvispora* studied in the present work agree with this view, being (12)13–13.5(14) × 6–6.5 (7) µm, Qm = 2.06.

Olariaga *et al.* (2015), Parslow & Spooner (2015) and Xu *et al.* (2018) reported that some existing names could be applied to the different species of the /alutacea clade, i.e. *Otidea cochleata* (L.) Fuckel, *O. felina* (Pers.) Bres., *O. alba* Velen., *O. cinerascens* Velen. and *O. kunmingensis* W.Y. Zhuang.

Otidea cochleata is here regarded as a *nomen dubium* following the discussions in Carbone (2010) and Parslow & Spooner (2013), as well as older authors like Nannfeldt (1966), Rifai (1968) and Dennis (1983).

Otidea alba and *O. felina* do not seem synonyms of *O. adorniae* because they produce much bigger ascospores, measuring (13.5–) 14.5–16.5(–17.5) × 6.5–7.5 µm (Olariaga *et al.*, 2015), and 14–16 × 6–6.5 µm / 15–17.5 × 6.5–7.5 µm (Van Vooren & Carbone, 2012; Parslow & Spooner, 2013, 2015), respectively.

Despite *O. cinerascens* was originally reported to have ascospores measuring 12 × 5–6 µm (Velenovský, 1947), we definitely found larger ascospores in the holotype, measuring 15.2 × 7.6 µm on average (see above). This is consistent with the notes on the herbarium sheet made by Spooner in 2009, reporting spores 14.5–16.5(–17) × 7–8.5 µm. Unfortunately, we were unable to produce any DNA sequence from the holotype, and so it is difficult to link this name to a specific genetic lineage. Further studies involving samples of

Plate 1 – *Otidea adorniae*. A: MCVE 30102 (Holotype); B: MCVE 30093; C-D: MCVE 30092; E: MCVE 30094; F: MCVE 30105. ***Otidea parvispora*.** G: MCVE 30108; F: MCVE 30107. Photos: A–E: C. Agnello; F: M. Carbone; G: J. Ruiz; F: G. Fransouas.

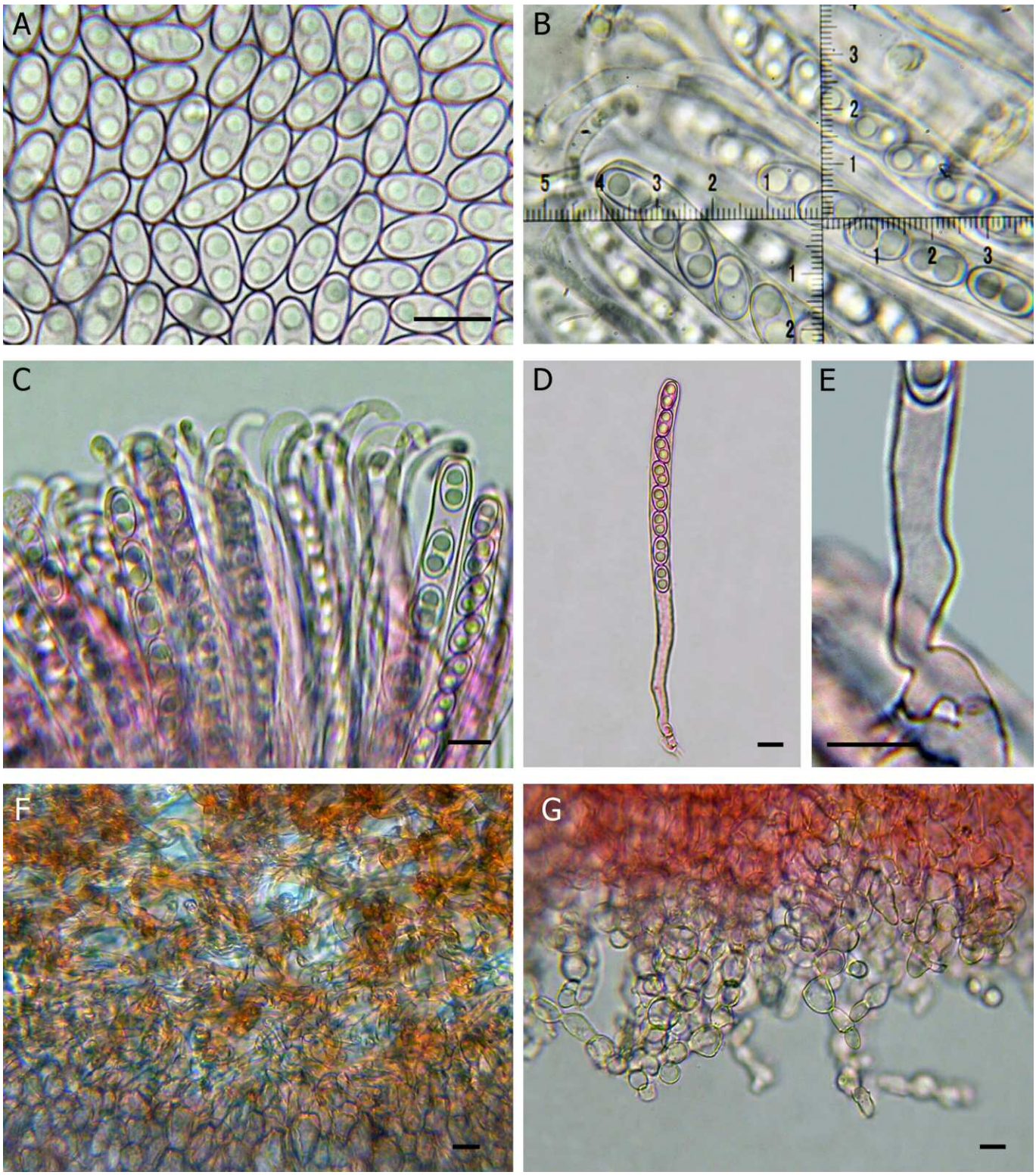

Plate 2 – *Otidea adorniae*. A: Ascospores; B–C: Asci and paraphyses; D: Ascus; E: Ascus pleurorynchous base; F: Ectal excipulum (bottom) and medullary excipulum; G: Terminal chained elements of the ectal excipulum. All mounted in Congo red except A and B in water. Bars = 10 µm. Photos C. Agnello.

Otidea aff. *alutacea* collected in southern Moravia, Czech Republic, are needed for a putative epitypification of *O. cinerascens*.

Otidea kunmingensis was first found in oak forests of Yunnan, China (Zhuang, 2008). This species resembles *O. adorniae* because of its morphology and ecology but has a definitely different genetic profile.

Otidea microspora (Kanouse) Harmaja (≡ *O. alutacea* var. *microspora* Kanouse) was proposed to accommodate North American collections with ascospores measuring 9–10 × 5.5–6.5 (7) µm, smaller than those of *O. alutacea* (Kanouse, 1949). In absence of an epitype, Parslow & Spooner (2015) selected a lectotype for *O. microspora* [Smith 17699 (MICH barcode 14406)], characterized by pale yellow apothecia, elements of the ectal excipulum encrusted by a yellow brown pigments, and ascospores 10–11.5 × 5.5–6.5 µm. They also stressed that the paratype "Smith 30502 (MICH02016)" was conspecific with the lectotype and both fit well the diagnosis of *O. alutacea* var. *microspora*. Olariaga *et al.* (2015) showed that the ITS rDNA sequence obtained from "Smith 30502" was identical to those of the types of *O. rainierensis* Kanouse and *O. kauffmanii* Kanouse, and therefore considered *O. microspora* a *nomen dubium*. This is also con-

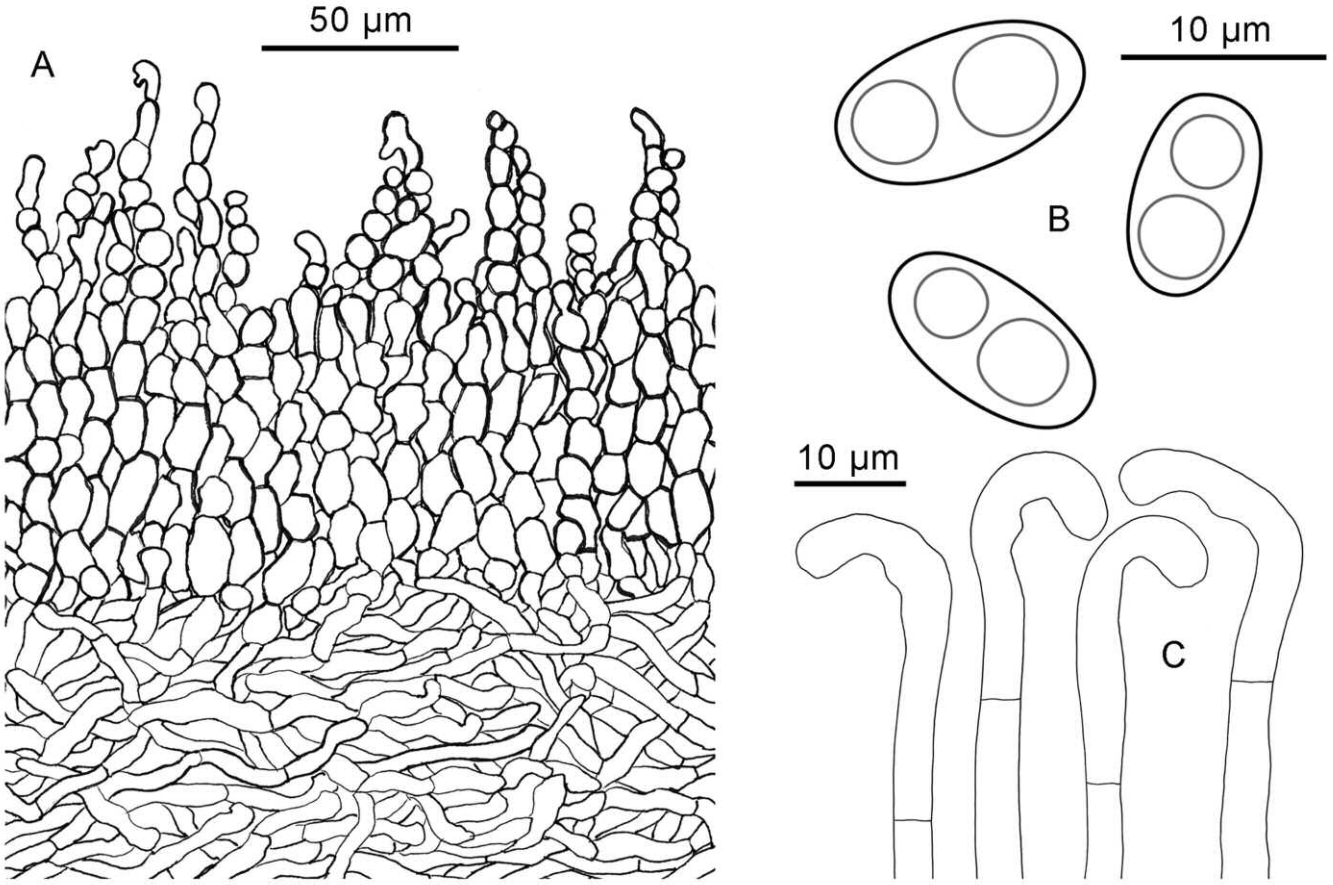

Fig. 3 – *Otidea adorniae*. Microscopical characters. A. Section; B. Ascospores; C. Paraphyses. Drawing C. Agnello.

sistent with results obtained by Peterson (1998) where "Smith 30502" was in the same clade of *O. rainierensis* and *O. kauffmanii*.

Additional collections and sequencing are needed to have a better knowledge on the real distribution of *O. adorniae*, so far reported only from Italy and United Kingdom [if we take into account the two ITS rDNA sequences published by Parslow & Spooner (2015)]. Lastly, a mention must be done about the putative *O. alutacea* (CUP-MM 2710) recorded in the Canary Islands by Korf & Zhuang (1991), a collection with ascospores measuring 9.5–11.7 × 5.9–7 µm which could maybe match *O. adorniae* or *O. parvispora*.

About the /bufonia clade

According to the genetic results reported above, the first important datum is that *Otidea pruinosa* Ekanayaka, Q. Zhao & K.D. Hyde (Hyde *et al.*, 2017) could be a synonym of *Otidea subpurpurea* W.Y. Zhuang, the latter genetically reviewed by Xu *et al*. (2018) along with *Otidea bicolor* W.Y. Zhuang & Zhu L. Yang.

As already shown by Carbone *et al*. (2017) and Xu *et al*. (2018), European samples of *Otidea bufonia* group in two distinct clades. At present no diagnostic morphological features have been detected to discriminate these two sister clades. A more detailed and extensive study is required in order to understand if the ecology may play a role on their reproductive isolation. These two clades are provisionally named here *O. bufonia* (1) and (2), waiting for further studies that can clarify the genetic identity of some putative synonym species, i.e.: *Otidea umbrina* (Pers.) Bres, *Peziza pseudobadia* Cooke and *Otidea pedunculata* Velen. (Olariaga *et al*., 2015).

Lastly, the presence of HMAS 23948 [identified as *Otidea olivaceobrunnea* Harmaja by Xu *et al.* (2018)] in the clade of *Otidea purpureogrisea* Pfister, F. Xu & Z.W. Ge suggests a very close relationship of both species, which needs to be better investigated.

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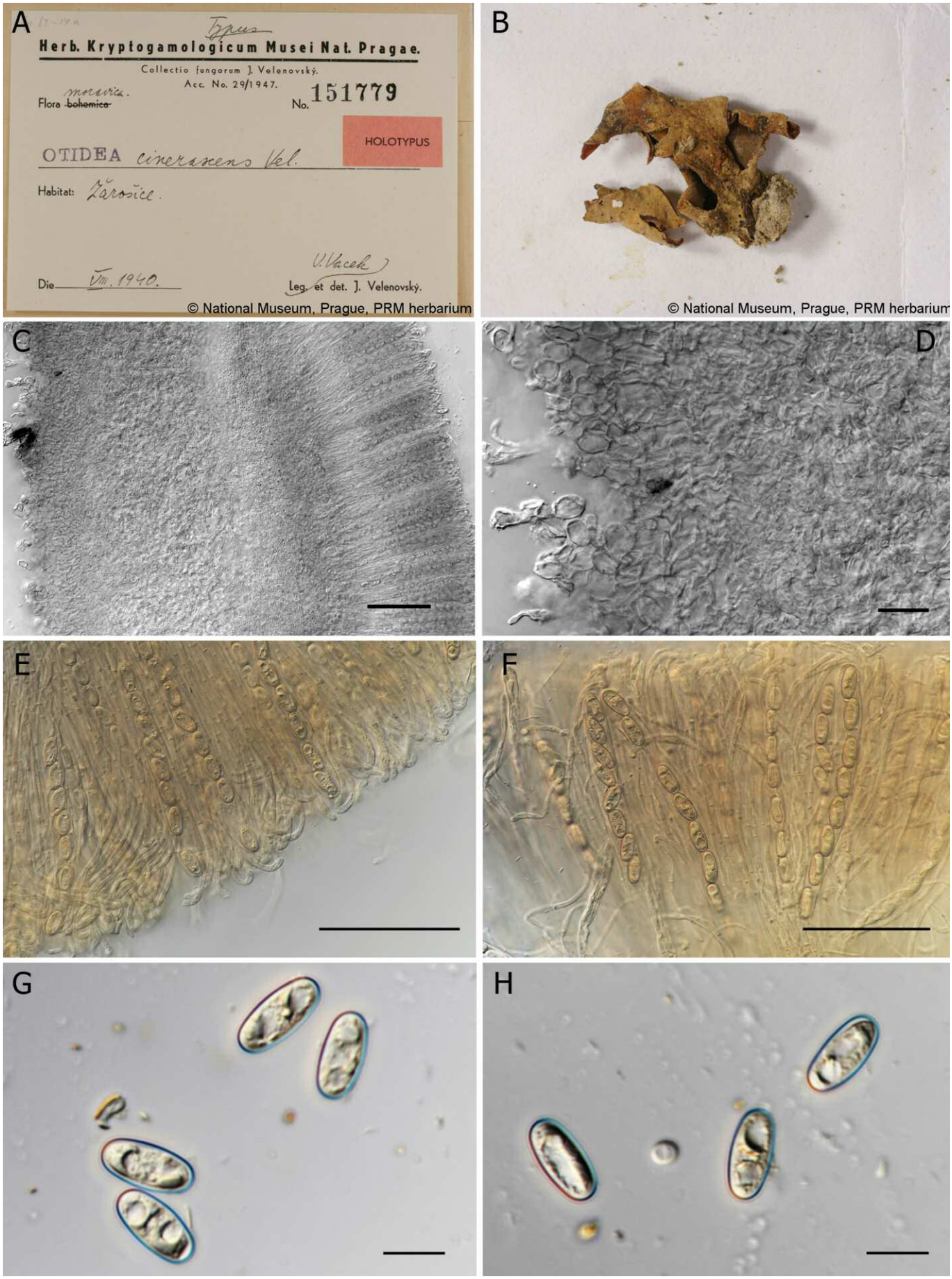

Plate 3 – *Otidea cinerascens* (holotype). A: Herbarium cover; B: Dried sample; C: Section; D: Medullary and ectal excipulum; E–F: Asci and paraphyses; G–H: Ascospores. Bars: C–F = 50 µm; H–I = 10 µm. Photos I. Kautmanová.

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