Warcupia cupulata a new cup-shaped species in the cleistothecioid genus *Warcupia* (*Otideaceae*, *Pezizales*)

Matteo CARBONE
Giovanni Battista GALEOTTI
Tomaso LEZZI
Antonis ATHANASIADIS
Pablo ALVARADO

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Abstract: The name *Warcupia cupulata* is here proposed for an apothecioid species described for the first time upon Greek, Italian and Spanish collections probably associated with *Cupressus*, and the formerly cleistothecioid genus *Warcupia* is consequently amended. Images of fresh samples and their main microscopical characters are provided, as well as phylogenetic analyses based on ITS rDNA sequences, and a combined dataset including 28S rDNA, tef1 and rpb2 data. Homologous sequences of the related genera *Acervus* and *Arpinia* were produced and published to support the phylogenetic study.
Keywords: *Acervus*, *Arpinia*, *Ascomycota*, phylogeny, *Pyronemataceae*, taxonomy.

Riassunto: Il binomio *Warcupia cupulata* viene qui proposto per una specie nuova apotecioide a seguito di raccolte effettuate in Grecia, Italia e Spagna probabilmente associate a *Cupressus*; conseguentemente viene emendato il genere cleistotecioide *Warcupia*. Vengono proposte foto di esemplari freschi e di microscopia, nonché due analisi filogenetiche basate sull'ITS solamente e su un allineamento combinato dei marcatori LSU, tef1 e rpb2. Infine, al fine di supportare lo studio filogenetico, sono state prodotte e pubblicate sequenze omologhe di specie appartenenti ai generi *Acervus* e *Arpinia*.
Parole chiave: *Acervus*, *Arpinia*, *Ascomycota*, filogenesi, *Pyronemataceae*, tassonomia.

Περίληψη: Η ονομασία *Warcupia cupulata* προτείνεται για ένα καινούργιο για την επιστήμη είδος με μορφή αποθήκιου, πιθανά συσχετιζόμενο με Κυπαρίσσια, που περιγράφεται από Ελληνικές, Ιταλικές και Ισπανικές συλλογές, και κατά συνέπεια το παλαιότερο γένος κλειστοθήκιων *Warcupia* τροποποιείται. Επισυνάπτεται εικονογραφία από φρέσκα δείγματα στο πεδίο μαζί με τα κύρια μικροσκοπικά χαρακτηριστικά τους, καθώς και φυλογενετικές αναλύσεις με βάση τόσο το ITS, όσο και συνδυασμένη ανάλυση των γονιδίων LSU, tef1 και rpb2. Ομόλογες ακολουθίες των σχετικών γενών *Acervus* και *Arpinia* δημοσιεύονται επιπρόσθετα για να υποστηρίξουν τη φυλογενετική μελέτη.
λέξεις-κλειδιά: *Acervus*, *Arpinia*, *Ascomycota*, *Pyronemataceae*, φυλογενετική ανάλυση, ταξινόμιση.

Introduction

The monotypic genus *Warcupia* Paden & J.W. Cameron, is typified by *Warcupia terrestris* Paden & J.W. Cameron, a species first isolated from soil samples of Canada and USA (Paden & Cameron, 1972). The genus is currently characterised by its globose brown cleistothecial ascocarps with a peridium composed of *textura angularis* with golden-brown cell walls, dehiscing at the apex at maturity to expose the unitunicate pleurorynchous asci, the hyaline, smooth, elliptical, and mainly biguttulate spores, and the paraphyses present during the entire development of the centrum, persisting at maturity (Paden & Cameron, 1972).

Paden & Cameron (1972) could not classify *Warcupia* in any given family with certainty, although they suggested it might belong to *Eoterfeziaceae* G.F. Atk. However, the authors recognised also that *Warcupia* could have derived from an operculate discomycete, since its particular developmental features do not exclude this possibility.

Warcupia was later transferred by Jeng & Krug (1976) into the tribe *Theleboleae* of the family *Pyronemataceae* Corda as defined by Korf (1972). On the contrary, Benny & Kimbrough (1980) considered *Warcupia* a member of the family *Eoterfeziaceae* (with a question mark). *Warcupia terrestris* has been recorded in Spain (Catalonia) by Fort & Guarro (1986) who described also *Warcupia terrestris* var. *minuta* Guarro. The new variety differs in its smaller spores and ascomata, the latter also maturing more slowly (Fort & Guarro, 1986).

Perry *et al.* (2007) were the first to notice that *Warcupia terrestris* is phylogenetically nested within the family *Pyronemataceae*, despite its cleistothecioid habit. Later, Hansen *et al.* (2013) reported that *Warcupia* is closely related to the highly reduced (gymnohymenial) genus *Monascella* Guarro & Arx, both forming a sister clade to the mainly apothecial genus *Otidea* (Pers.) Bonord. This latter classification was accepted by Jaklitsch *et al*. (2016) and confirmed by Ekanayaka *et al.* (2018) who considered that Warcupia belongs to the family *Otideaceae* Eckblad together with the genera *Acervus* Kanouse, *Arpinia* Berthet, *Ascosparassis* Kobayasi, *Diehliomyces* Gilkey, *Monascella*, *Otidea*, and *Wenyingia* Zheng Wang & Pfister.

During the last two years we have collected in Greece and Italy an apothecioid species showing a mix of features recalling the family *Otideaceae* but not completely matching any of the known genera. At first sight, its habit resembles that of the genera *Tarzetta* (Cooke) Lambotte or *Arpinia*, but microscopical features do not fit these genera. Surprisingly, the preliminary genetic results obtained from ITS, LSU, tef1 and rpb2 markers suggested a close relationship with *Warcupia terrestris*. An additional collection from Spain has been genetically confirmed during the final review of the paper.

The aim of the present article is to resolve the most suitable taxonomic treatment for these collections, comparing them morphologically and genetically with the most similar species of *Otideaceae*.

Material and methods

Morphological study. — The microscopic studies were based on both fresh and dried specimens. The following optical microscopes were used: Optika B-150 with Optika B1 (digital camera), Optika B510BF with Optikam C-P6, Nikon Eclipse E400 with Moticam 580, with plan-achromatic objectives 10×, 20×, 40×, 60×, 100× oil immersion. The following main reagents were used: Melzer's reagent to test the amyloid reaction, cotton blue to check the ascospores surface, Congo red to highlight the micro-structures, 5% KOH and water to rehydrate the dried material. In addition, water and L4 mounts were used for observation of the pigmentation and measurements. Measurements were taken using the software Piximètre.

DNA extraction, amplification and sequencing. — Total DNA was extracted from dry specimens employing a modified protocol based on Murray & Thompson (1980). PCR reactions (Mullis & Faloona, 1987) included 35 cycles with an annealing temperature of 54 °C. The primers ITS1F and ITS4 (White *et al*., 1990; Gardes & Bruns, 1993) were employed to amplify the ITS rDNA region, LR0R and LR5 (Vilgalys & Hester, 1990; Cubeta *et al*., 1991) were used for the 28S rDNA region, EF1-728F, EF1-983F and EF1- 2218R (Carbone & Kohn, 1999; Rehner & Buckley, 2005) for the translation elongation factor 1a (tef1) gene, and bRPB2-6F2 (reverse of bRPB2-6R2), and bRPB2-7R2 for the RNA polymerase II second largest subunit (rpb2) gene (Matheny *et*

al., 2007). PCR products were checked in 1% agarose gels, and amplicons were sequenced with one or both PCR primers. Sequences were corrected to remove reading errors in chromatograms.

Phylogenetic analyses. — Two independent analyses were done employing: 1) a combined dataset of LSU, tef1 and rpb2 data (excluding introns) from selected samples of the family *Otideaceae* (with samples from the family *Sarcosomataceae* as outgroup), and 2) an independent analysis of ITS sequences of *Otideaceae* (again with *Sarcosomataceae* as outgroup). BLASTn (Altschul *et al.*, 1990) was used to select the most closely related sequences from the International Nucleotide Sequence Database Collaboration public database (INSDC, Cochrane *et al.*, 2011) and UNITE (Nilsson *et al.*, 2018). The sequences retrieved (Tab. 1) were published in many different works, the largest number produced by Hansen & Olariaga (2015) and Zeng *et al.* (2020). Sequences first were aligned in MEGA 5.0 (Tamura *et al.*, 2011) with its Clustal W application and then realigned manually as needed to establish positional homology. The final alignments included 291/456/57 (ITS), 243/720/29 (LSU), 335/865/26 (tef1) and 299/589/26 (rpb2) variable sites/total sites/sequences. The two independent datasets were loaded in MrBayes 3.2.6 (Ronquist *et al.*, 2012) and subjected to Bayesian analysis (LSU, tef1 and rpb2 analysed in separate partitions, GTR+G+I model for all partitions, two simultaneous runs, four chains, temperature set to 0.2, sampling every 100th generation) until the average split frequencies between the simultaneous runs fell below 0.01 after 1.34 M (ITS) and 0.06 M (LSU-tef1-rpb2) generations. Finally, a full search for the best-scoring maximum likelihood tree was performed in RAxML 8.2.12 (Stamatakis, 2014) using the standard search algorithm (same partitions, GTRGAMMAI model, 2000 bootstrap repli-

Tab. 1 – Sequences used for this study and GenBank accession numbers, in bold those newly generated. (T) means "Type".

Herbarium Number	Species	ITS	LSU	rpb2	tef1
HMAS:78150 (T)	*Acervus beijingensis*	–	NG05697	KP993484	KP993492
HMAS:78146 (T)	*Acervus changchunensis*	–	NG057068	KP993482	KP993490
TUR-A:209466	***Acervus epispartius***	**MW884544**	**MW884555**	**MW892390**	**MW892385**
MFLU:20-0257	*Acervus epispartius*	–	MT165625	MT210807	MT336154
HKAS:90046	*Acervus flavidus*	–	KX765259	MG980718	KX765252
HKAS:88987 (T)	*Acervus globulosus*	NR159565	–	–	
HKAS:88987	*Acervus globulosus*	MG871290	NG057117	MG980715	KX765255
HKAS:88987	*Acervus globulosus*	–	MT165626	MT210808	MT336155
HMAS:271281 (T)	*Acervus heilongjiangensis*	–	NG057059	KP993485	KP993493
MFLU:20-0259	*Acervus rufus*		–	MT210811	MT227372
FLAS:F-63813	*Acervus* sp.	MT156531	–	–	–
MFLU:20-0260	*Acervus stipitatus*	–	MT165627	MT210809	MT336156
MFLU:16-0607 (T)	*Acervus stipitatus*	–	NG057118	KX765257	KX765258
GDOR:1020	***Arpinia inops***	**MW884545**	**MW884556**	–	–
C:HD Rana 75.082	*Arpinia inops*	–	DQ220315	–	–
GDOR:2950	***Arpinia luteola* var. *pallidorosea***	**MW884546**	**MW884557**	**MW892391**	**MW892386**
?	*Diehliomyces microsporus*	AY839842	–	–	–
K:M136920	*Diehliomyces microsporus*	EU784196	–	–	–
CBS:135.92	*Galiella rufa*	AF485070	FJ176869	FJ238352	FJ238401
FH:PR6376	*Glaziella aurantiaca*	–	KC012681	JX943754	KC109242
CBS:233.85 (T)	*Monascella botryosa*	NR145208	NG066259	JX943831	KC109256
MCVE:30102 (T)	*Otidea adorniae*	NR164497	–	–	–
MC201009 (T)	*Otidea borealis*	KM010023	–	–	–
S:KH.08.107 (T)	*Otidea brunneoparva*	NR158811	–	–	–
S:KH.09.172	*Otidea bufonia*	–	JN941097	KM823397	KM823272
H:6010805	*Otidea caeruleopruinosa*	KF717575	–	–	–
KH.09.125	*Otidea cantharella*	KM010084	–	–	–
KH.09.183 (T)	*Otidea concinna*	KM010032	–	–	–
S:KH.09.250	*Otidea concinna*	–	JN941095	JN993544	KM823276
AM-AR17-016	*Otidea daliensis*	MH930311	–	–	–
H:6003549	*Otidea formicarum*	KF717577	–	–	–
Z.W. Ge 1913 (T)	*Otidea korfii*	KU987017	–	–	–
HKAS:49452 (T)	*Otidea kunmingensis*	NR164498	–	–	–
H:6003548	*Otidea leporina*	KF717578	–	–	–
S:F256929 (T)	*Otidea mirabilis*	NR120289	–	–	–
H:6002902 (T)	*Otidea nannfeldtii*	NR120290	–	–	–
S:KH.10.284 (T)	*Otidea onotica*	NR158814	NG060314	KM823429	KM823299
OSC:Moorefun 58 (T)	*Otidea oregonensis*	NR155563	–	–	–
H:6003547 (T)	*Otidea papillata*	KF717582	KM823234	KM823435	KM823305

Tab. 1 (continued)

Herbarium	Species	ITS	LSU	rpb2	tef1
K:M124712	*Otidea platyspora*	EU784393	–	–	–
HKAS:81819 (T)	*Otidea pruinosa*	NR155604	–	–	–
Z.W. Ge 863	*Otidea purpureogrisea*	KU987011	–	–	–
MICH:14410 (T)	*Otidea rainierensis*	NR120292	–	–	–
ISC:445962 (T)	*Otidea subterranea*	–	FJ404767	–	–
H:6002901 (T)	*Otidea tuomikoskii*	NR120293	–	–	–
CBS:143165 (T)	*Planamyces parisiensis*	MG386040	MG386093	MG386141	–
TUR-A:195784	*Plectania melastoma*	JX669814	–	–	–
FH:KH.97.16	*Plectania nannfeldtii*	–	AY945853	DQ017592	KC109214
MCVE:27396	*Pseudoplectania nigrella*	KF305715	–	–	–
S:KH.07.04	*Sarcosoma globosum*	KC109215	–	–	–
UAMH:1497	*Sporendonema purpurascens*	GQ272631	–	–	–
KACC:41227	*Sporendonema purpurascens*	GQ272632	–	–	–
CBS:406.63	*Sporendonema purpurascens*	MH858317	–	–	–
ectomycorrhiza *Larix*	undetermined	GU181839	–	–	–
air sample	undetermined	HQ115650	–	–	–
twigs of *Olea*	undetermined	KT804119	–	–	–
twigs of *Olea*	undetermined	KT804149	–	–	–
soil sample	undetermined	MF971812	–	–	–
soil sample	undetermined	UDB0238227	–	–	–
soil sample	undetermined	UDB0265435	–	–	–
soil sample	undetermined	UDB0335870	–	–	–
soil sample	undetermined	UDB0491370	–	–	–
soil sample	undetermined	UDB0626295	–	–	–
soil sample	undetermined	UDB065905	–	–	–
soil sample	undetermined	UDB0683114	–	–	–
soil sample	undetermined	UDB0769576	–	–	–
TUR-A:195794	*Urnula craterium*	JX669820	–	–	–
FH:DHP04-511	*Urnula craterium*	–	AY945851	DQ017595	KC109216
TUR-A:209462	***Warcupia cupulata***	**MW884549**	**MW884560**	–	**MW892389**
TUR-A:209465	***Warcupia cupulata***	**MW884548**	**MW884559**	**MW892393**	**MW892388**
TUR-A:209464 (T)	***Warcupia cupulata***	**MW884547**	**MW884558**	**MW892392**	**MW892387**
CBS:131.84 (T)	*Warcupia terrestris* var. *minuta*	MH861708	–	–	–
CBS:891.69 (T)	*Warcupia terrestris* var. *terrestris*	MH859473	MH871254	–	–

cations). The significance threshold was set above 0.95 for posterior probability (PP) and 70% bootstrap proportions (BP).

Studied and/or sequenced collections

Warcupia cupulata. Greece. Thessaly, Elaiones (suburb) of Thessaloniki, 40° 35′ 46.21″ N, 23° 1′ 4.47″ E, alt. 185 m, on calcareous soil under *Cupressus sempervirens* f. *horizontalis* and *Pinus brutia*, 16.XII.2019, *leg*. A. Athanasiadis (TUR-A 209462). Italy. Umbria, Città di Castello (PG), 43° 27′ 8.8″ N 12° 13′ 24.1″ E, alt. 400 m, in the moist soil of a private park under mixed broad-leaved and conifer trees, 08.XII.2019, *leg*. G. Galeotti (TUR-A 209463). *Ibidem*, 05.XII.2020, *leg*. G. Galeotti (TUR-A 209464, holotype). *Ibidem*, 08.I.2021, *leg*. G. Galeotti & T. Lezzi (TUR-A 209465). Spain. Extremadura, Cáceres, Casas del Miravete, 39° 44′ 18.58″ N 5° 45′ 4.34″ W, alt. 371 m, on soil under *Eucalyptus camaldulensis* and *Cupressus* sp., 27.II.2021, *leg*. J.A. Suárez, *rev*. E. Rubio (ERD-8749), GenBank codes ITS: MZ501736, LSU: MZ501802. ***Acervus epispartius***. Italy. Veneto, Belluno, 46° 9′ 0.2″ N 12° 11′ 20.5″ E, alt. 550 m, under mixed broad-leaved trees, 27.IX.2020, *leg*. F. Padovan (TUR-A 209466). ***Arpinia inops***. Italy. Trentino Alto Adige, Badia (BZ), between Pedraces and Pescol, 46° 37′ 6.6″ N 11° 53′ 22.9″ E, alt. 1360 m, under *Picea abies*, 11.VII.2009, *leg*. F. Boccardo (GDOR 1020). ***Arpinia luteola* var. *pallidorosea***. Italy. Liguria, Sassello (SV), fraz. Badani, 44° 28′ 24.9″ N 8° 29′ 14.6″ E, alt. 300 m, under broad-leaved trees, 08.VI.2013, *leg*. F. Boccardo (GDOR 2950).

Phylogenetic results

The combined phylogenetic analysis of LSU, rpb2 and tef1 sequences of *Otideaceae* (Fig. 1) resulted in four significantly supported major clades: genus *Otidea*, genus *Arpinia*, genus *Acervus*, and another significantly monophyletic lineage including *Monascella*, *Planamyces* Crous & Decock, *Warcupia* and three samples analysed in the present study. The analysis of ITS data (Fig. 2) could not recover a significant support for the clade of *Monascella*, *Planamyces* and *Warcupia*, where ITS sequences identified as *Sporendonema purpurascens* (Bonord.) E.W. Mason & S. Hughes nested too. The genus *Sporendonema* is typified by *S. casei* Desm., which apparently belongs in the order *Onygenales* (Ropars *et al*., 2012), so this generic name should not be employed for this clade. Finally, ITS sequences identified as *Diehliomyces microsporus* (Diehl

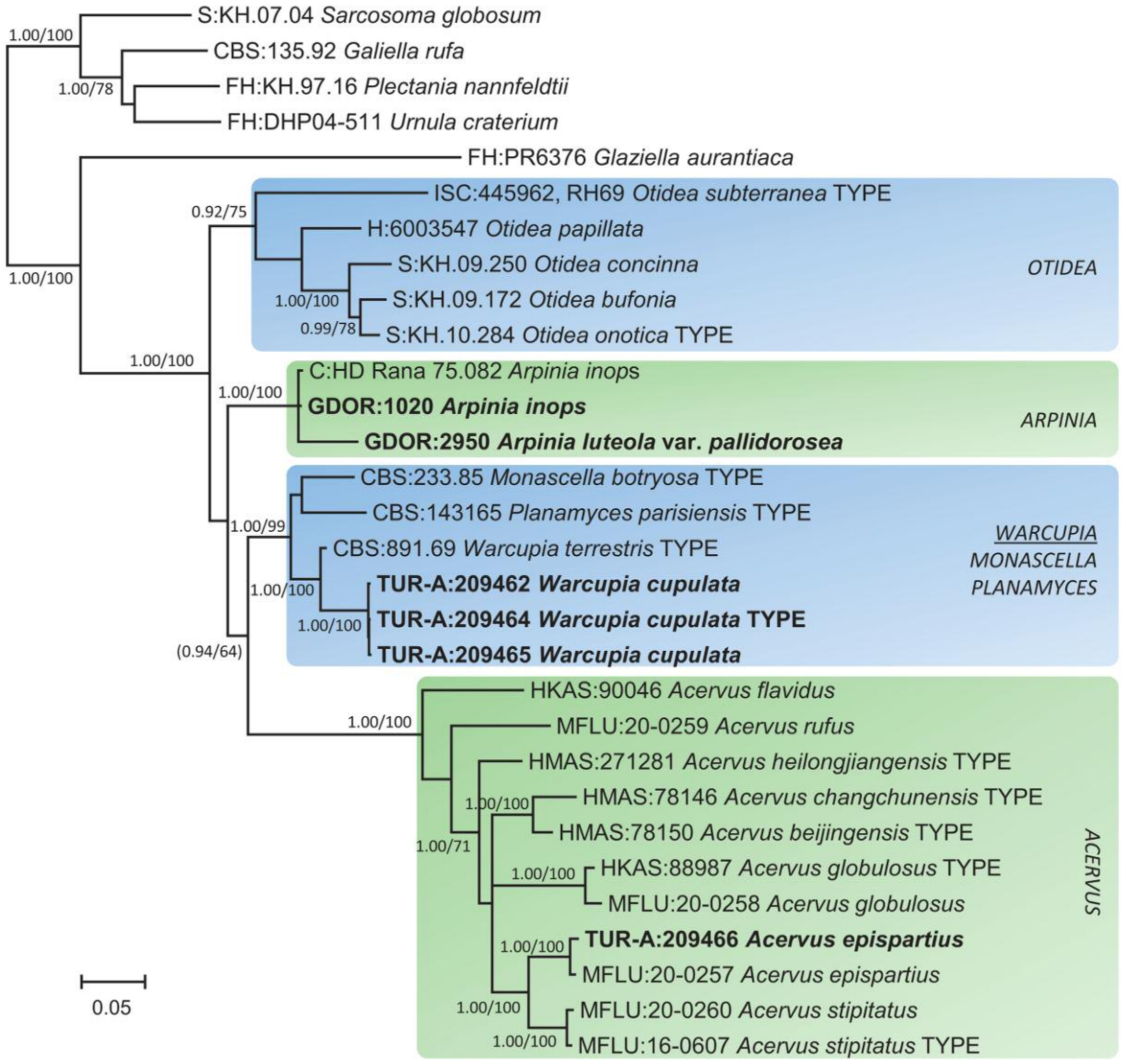

Fig. 1 – 50% majority rule 28S rDNA - tef1- rpb2 consensus phylogram of the family *Otideaceae* (*Pezizales*) (with selected species of the family *Sarcosomataceae* as outgroup) obtained using MrBayes from 450 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Sequences newly generated in this study are in bold.

& E.B. Lamb.) Gilkey (type species of *Diehliomyces*) were the closest match to the sample of *Acervus epispartius* analysed in the present work.

These results suggest that a number of taxonomic changes could be necessary: 1) synonymise *Acervus* and *Diehliomyces*, combining the species of the former into *Diehliomyces*, 2) synonymise *Monascella* and *Planamyces* with *Warcupia*, moving all species to *Warcupia*, 3) review the status of samples identified as *Sporendonema purpurascens* and eventually combine this species into *Warcupia* or propose a new name for these collections, and 4) propose a new species of *Warcupia* for the samples related to this genus analysed in the present work. Most of these important changes require further data to be adequately supported, and so, the only task accomplished in the present work is the proposal of a new species related to *Warcupia terrestris*.

Taxonomy

Warcupia Paden & J.V. Cameron, *Canad. J. Bot.*, 50(5): 999 (1972).

Type species: *Warcupia terrestris* Paden & J.V. Cameron, *Canad. J. Bot.*, 50(5): 999 (1972).

Amended description: Ascomata cleistothecioid or apothecioid, small or medium sized, subglobose or cupuliform with a more or less well-developed stipe, brownish or yellow-brown. Ascospores ellipsoid, hyaline, smooth, biguttulate. Asci operculate, narrowing toward base, arising from croziers, inamyloid, 8-spored. Paraphyses cylindrical, hyaline, tips straight or curved. Ectal excipulum of *textura angularis* or *globulosa/angularis*. Medullary excipulum present only in the apothecioid species, of *textura intricata*. Asexual morph unknown.

Warcupia cupulata M. Carbone, Galeotti, Lezzi, Athanasiadis & P. Alvarado, *sp. nov.* – MB 839205 – Pl. 1–5

Diagnosis: Besides its characteristic genetic profile, *Warcupia cupulata* differs from the only other known species of this genus, *W. terrestris*, in forming apothecia and having smaller ascospores. It differs also from the entirely-cupulate species of the genus *Otidea* in having smaller ascospores, from species of *Arpinia* in the curved tips of the paraphyses, and from species of *Tarzetta* in its pleurorynchous asci.

Holotype here designated: Italy. Umbria, Città di Castello (PG), in the moist soil of a private park under mixed broadleaved and conifer trees, 05.XII.2020, *leg.* G. Galeotti, TUR-A 209464.

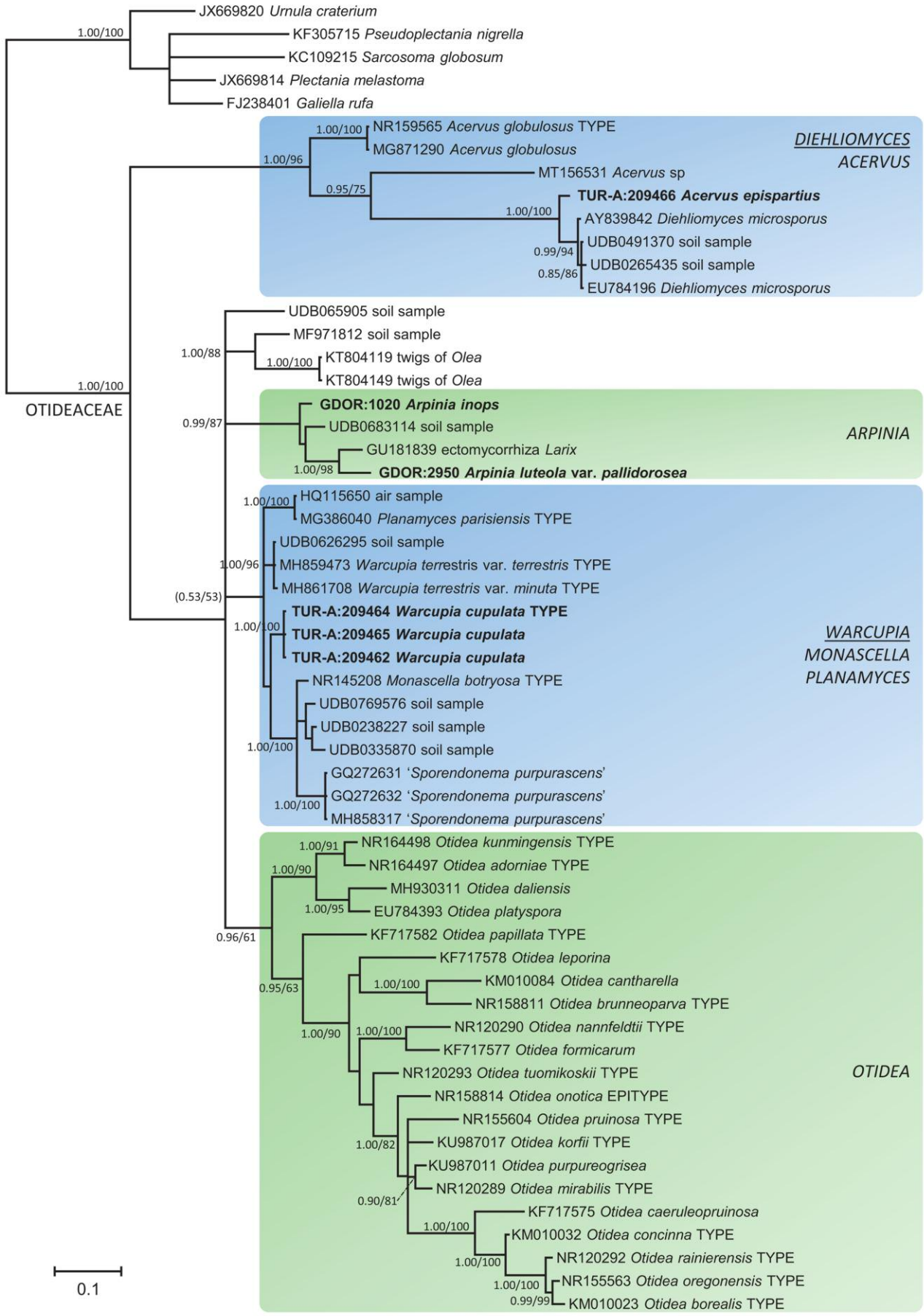

Fig. 2 – 50% majority rule ITS rDNA consensus phylogram of the family *Otideaceae* (*Pezizales*) (with selected species of the family *Sarcosomataceae* as outgroup) obtained using MrBayes from 450 sampled trees. Nodes were annotated if they were supported by ≥ 0.95 Bayesian posterior probability (left) or ≥ 70% maximum likelihood bootstrap proportions (right). Sequences newly generated in this study are in bold.

Etymology: *cupulata*, from the latin *cupulatus* [*a*, *um*], meaning cup-shaped, because of its habit.

Macroscopical features (plates 1 and 3)

Ascomata gregarious to fasciculate. **Apothecia** 5–30 mm wide, 20–40 mm high, stipitate, disc entire although in a few samples a lateral otideoid split was present. **External surface** pubescent, pruinose to furfuraceous, light hazelnut brown with orangish tints when young, darker at complete maturity. **Margin** involute and finely crenulate in young specimens, then denticulate and decorated with rusty warts. **Hymenium** smooth, subconcolorous with the external surface but with greyish tints in youth, rugulose to wrinkled and with brown-purplish tints at maturity. **Stipe** well developed, thick, solid in section, vertically wrinkled with wrinkles embracing the cup base for a short distance. **Flesh** with indistinct taste or smell. **Basal mycelium** whitish.

Microscopic features (plates 2–5)

Ascospores smooth, hyaline, ellipsoid to slightly ovoid or inequilateral, (8.8–)9.5–11(–11.8) × (4.9–)5.6–6.7(–7.3) µm, Q = (1.4–)1.5–1.9(–2.0), average = 10.3 × 6.1 µm, Qm = 1.7 (N = 161), biguttulate, with wall up to 0.4 µm thick. **Paraphyses** cylindrical, septate, branched at the base, slightly longer than the asci, c. 2.2 µm wide, tips mostly bent to hooked, enlarged up to 2.5 µm wide, some with a low notch on the underside. **Asci** cylindrical, (113.6–)129.5–152.4 (–227.4) × (6.7–)7.8–9.3(–9.5) µm, Q = (12.7–)14.8–19.5(–26.3), average = 144.6 × 8.4 µm, Qm = 17.3 (N = 30), 8-spored, inamyloid, operculate, pleurorhynchous. **Subhymenium** visible as a slightly darker small zone, composed of densely arranged cylindrical cells. **Medullary excipulum** arranged as a *textura intricata*, composed of hyaline hyphae measuring (6.8–)8.1–13(–17.5) µm wide, average = 10.4 µm (N = 30), thin-walled. **Ectal excipulum** of *textura globulosa-angularis* in the inner part, with cells slightly thick-walled, hyaline to very pale yellowish, (8.7–)9.6–13.7(–16.2) µm in diam., average = 10.4 µm (N = 30); in the outer part (i.e. the warts) composed of cells arranged as *textura globulosa* to *globulosa-angularis*. **Basal mycelium** composed by hyaline, septate hyphae, 8.5 µm wide, wall up to 1 µm thick, mostly smooth.

Ecology, phenology and distribution: The known collections fruited from November to February in one locality in Greece (Thessaly), two in Italy (Umbria) and one in Spain (Extremadura). Greek samples were collected in the soil of a conifer forest of *Pinus brutia* and *Cupressus sempervirens* f. *horizontalis*. The holotype and other Italian collections were found at 400 m a.s.l. in a private park, in an area where multiple tree species (some of them allochthonous) can be found very close to each other, i.e. *Cupressus lusitanica*, *C. macrocarpa*, *Quercus ilex*, *Picea orientalis*, *P. abies*, *Abies cephalonica*, *A. alba*, *A. pinsapo*, *Taxus baccata*, and *Calocedrus decurrens*. During the final review of the present study, we were informed of: i) another Italian collection recorded in Tuscany, found under a typical tree-lined Tuscan avenue of *Cupressus macrocarpa*; ii) a Spanish collection found under *Eucalyptus* and *Cupressus* sp. Since all known collections of *W. cupulata* were found near *Cupressus* spp., it is possible that it is biologically linked to this genus or at least to the habitats where it is present.

Unfortunately, the exact ecology of the other known species *Warcupia terrestris* is still unknown (Paden & Cameron, 1972; Fort & Guarro, 1986) and so an ecological correlation between these two species is difficult to find.

Discussion

As reported in the introduction, *Warcupia* was established for the cleistothecioid species *Warcupia terrestris*. The present phylogenetic results show that sequences of the apothecioid species named here *Warcupia cupulata* are significantly similar to those of *W. terrestris*, suggesting a monophyletic origin of both taxa. Besides their macromorphological differences, these two species share some common features, e.g. smooth biguttulate spores and pleurorhynchous asci. Recently, Van Vooren *et al.* (2021) noticed that the cleistothecioid genus *Lasiobolidium* Malloch & Cain is probably a synonym of the apothecioid genus *Paratrichophaea* Trigaux. In addition, in the related genus *Otidea* we can find also a sequestrate truffle-like species, *Otidea subterranea* R.A. Healy & M.E. Sm. (Smith & Healy, 2009; Olariaga *et al.*, 2015). According to the present results, the generic concept of *Warcupia* is amended to accommodate the cup-shaped species *W. cupulata*.

Warcupia cupulata is easily recognizable by the cupulate-stipitate apothecium resembling species of *Arpinia* (see e.g. Berthet, 1974; Benkert, 1980; Hohmeyer, 1988) but with a microscopical framework definitely matching more that of the genus *Otidea* (Olariaga *et al.*, 2015). However, the combination of macro- and microscopical features seems not to fit any of the known species of *Arpinia* and *Otidea*, although asci and ascospores are somewhat similar.

Arpinia species apparently lack bent or hooked paraphyses tips, and in most species, ascospores are longer and/or wider than in *Warcupia cupulata* (Hohmeyer, 1988). Ascospores of a similar size can be found only in *Arpinia luteola* J. Geesink, *A. luteola* var. *pallidorosea* Benkert, Häffner & Hohmeyer and *A. microspora* (Dissing & Raitv.) Hohmeyer. *Arpinia luteola* and its var. *pallidorosea* have different colours (yellowish orange and cream to pinkish respectively), paraphyses with straight tips and slightly narrower ascospores, being 9.5–11.5 × 5.5–6.0 µm (Geesink, 1982, for *A. luteola*), (9.5–)10–12(–13) × (4.5–)5–6 µm and (9.5–)10–11(–11.5) × (4.5–)5–6(–6.5) µm (Hohmeyer, 1988, for *A. luteola* and var. *pallidorosea*), (9–)9.6–11.2 (–12) × (5.2–)5.6–6 µm (Fernàndez Vicente & Undagoitia, 2004), and 10–12 × 5.5–6 µm (Moyne, 2008). *Arpinia microspora* differs in its smaller, whitish, subsessile apothecia, the straight tips of its paraphyses, and its slightly longer and wider ascospores, 10.5–11.9–12.6 × 6.3–6.9–8.0 µm (Dissing & Raitviir, 1974) and 10.5–12.5(–13) × 6.5–7.5(–8) µm (Hohmeyer, 1988).

Distinctly-stipitate species of *Otidea* with entire cup, such as *Otidea propinquata* (P. Karst.) Harmaja and *O. phlebophora* (Berk. & Broome) Sacc. differ in many respects from *Warcupia cupulata*. *O. propinquata* has ochre-brown to reddish-brown colors, bigger ascospores, (18–)19–21 × 10–12.5 µm, and notched or forked paraphyses (see among others Carbone, 2010; Olariaga *et al.*, 2015). *O. phlebophora* differs in its pale ochre hymenium (sometimes with rose stains), the bright citrine yellow color of the external surface, and narrower ascospores, Wm = 4.9-5.1 µm, with a larger Q (Qm = 2-2.1) (Olariaga *et al.*, 2015). *Warcupia cupulata* seems also to be different from *Otidea* species in lacking an evident encrusting pigment of the ectal excipular cells.

Ekanayaka *et al.* (2018) consider that *Wenyingia sichuanensis* Zheng Wang & Pfister, a Chinese species morphologically similar to the genus *Tarzetta* (Wang & Pfister, 2001), belongs also to the family *Otideaceae*, but without molecular support. The microscopical features of this genus are very different from those of *Warcupia cupulata*, so we here exclude that this species could belong to *Wenyingia*. In any case, genetic data of *W. suchuanensis* are needed to confirm its systematics, although the absence of croziers strongly suggests it could belong to *Tarzettaceae*.

Genetically, the LSU rDNA sequences of *W. cupulata* are 97.3% similar to those of *W. terrestris*, a value slightly below the average optimal threshold between genera (98.21%, Vu *et al.*, 2019). The distances with *Planamyces* (96.0%) and *Monascella* (95.4%) are also below this value, but the average similarity of LSU rDNA observed between species of other genera of *Otideaceae* is even lower than the proposed threshold between genera: 94.6% between species of *Acervus*, and 90.7% between species of *Otidea*. Regarding ITS rDNA, the distance between *W. terrestris* and *W. cupulata* is 95.8% slightly above the average threshold between genera (94.31%), while the average distance between ITS rDNA of the species of *Otidea* analysed in the present work is about 75%. In this context,

Plate 1 – *Warcupia cupulata*. Macroscopical habit.
A–B: (TUR-A 209465, T. Lezzi); H: (TUR-A 209464, G. Galeotti); all the remaining pictures (TUR-A 209463, G. Galeotti).

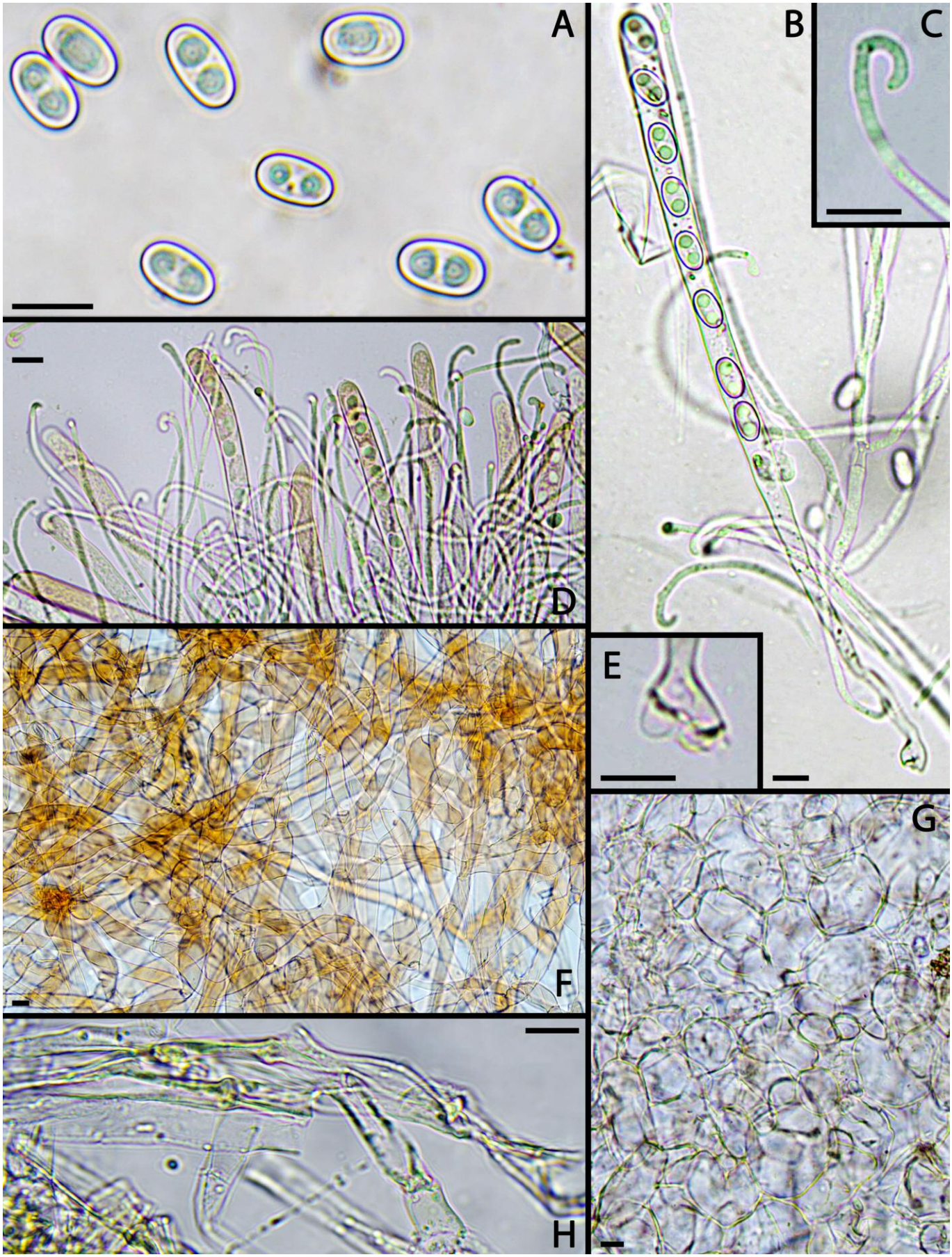

Plate 2 – *Warcupia cupulata*. A: ascospores; B: ascus and paraphyses; C: tips of paraphyses; D: upper part of asci and paraphyses; E: ascus base; F: medullary excipulum; G: ectal excipulum; H: basal mycelium. A, D, F in Congo Red mounts; B, C, E, G, H in water mounts. Scale bars = 10 µm. Photos G. Galeotti.

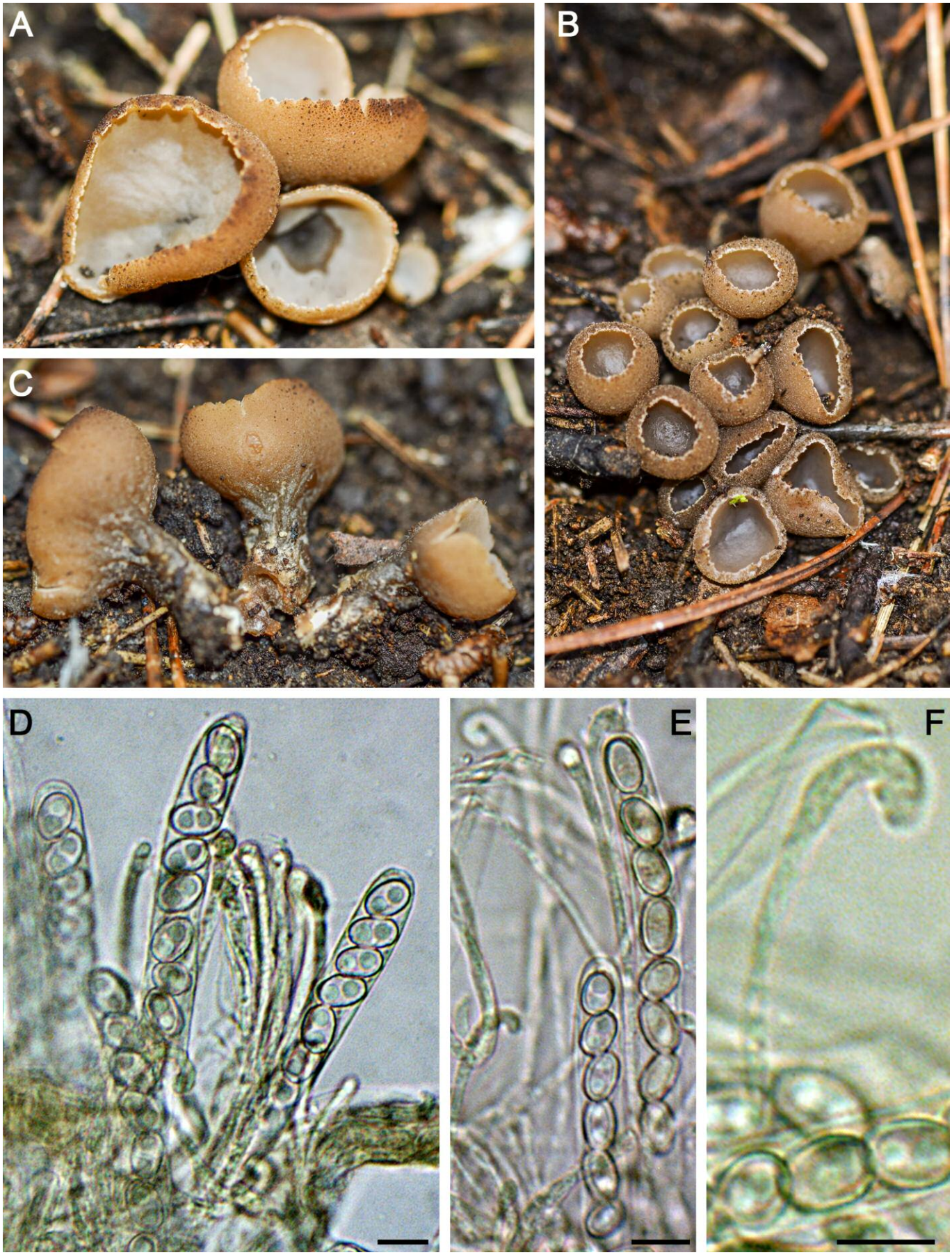

Plate 3 – *Warcupia cupulata*. TUR-A 209462. A–C: ascomata *in situ*; D-E: asci, ascospores and paraphyses tips in water mount; F: paraphyses tip in water mount. Scale bars = 10 µm. Photos A. Athanasiadis.

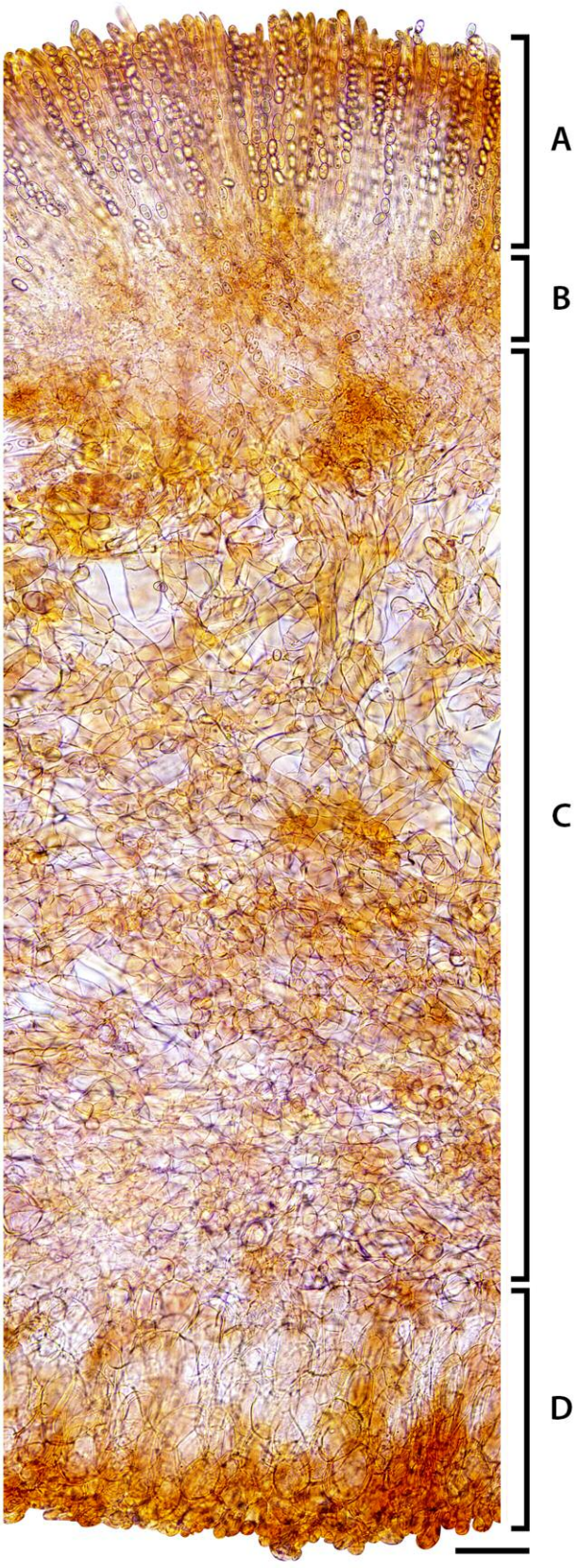

Plate 4 – *Warcupia cupulata*. Section of the apothecium in Congo Red mount. A: asci and paraphyses; B: subhymenium; C: medullary excipulum; D: ectal excipulum. Scale bar = 50 µm. Photo G. Galeotti & T. Lezzi.

classifying the newly described species inside *Warcupia* is here considered, by now, the best taxonomic option.

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Authors' contributions

Matteo Carbone was responsible for the study conception. M. Carbone, G. Galeotti, T. Lezzi and A. Athanasiadis financially contributed to the generation of rDNA sequences. Morphological analyses were performed by the authors on their collections. Molecular analyses and all the phylogenetic chapters and notes were performed by P. Alvarado (ALVALAB). The first draft of the manuscript was written by M. Carbone and was subsequently updated by all authors. All authors read and approved the final manuscript.

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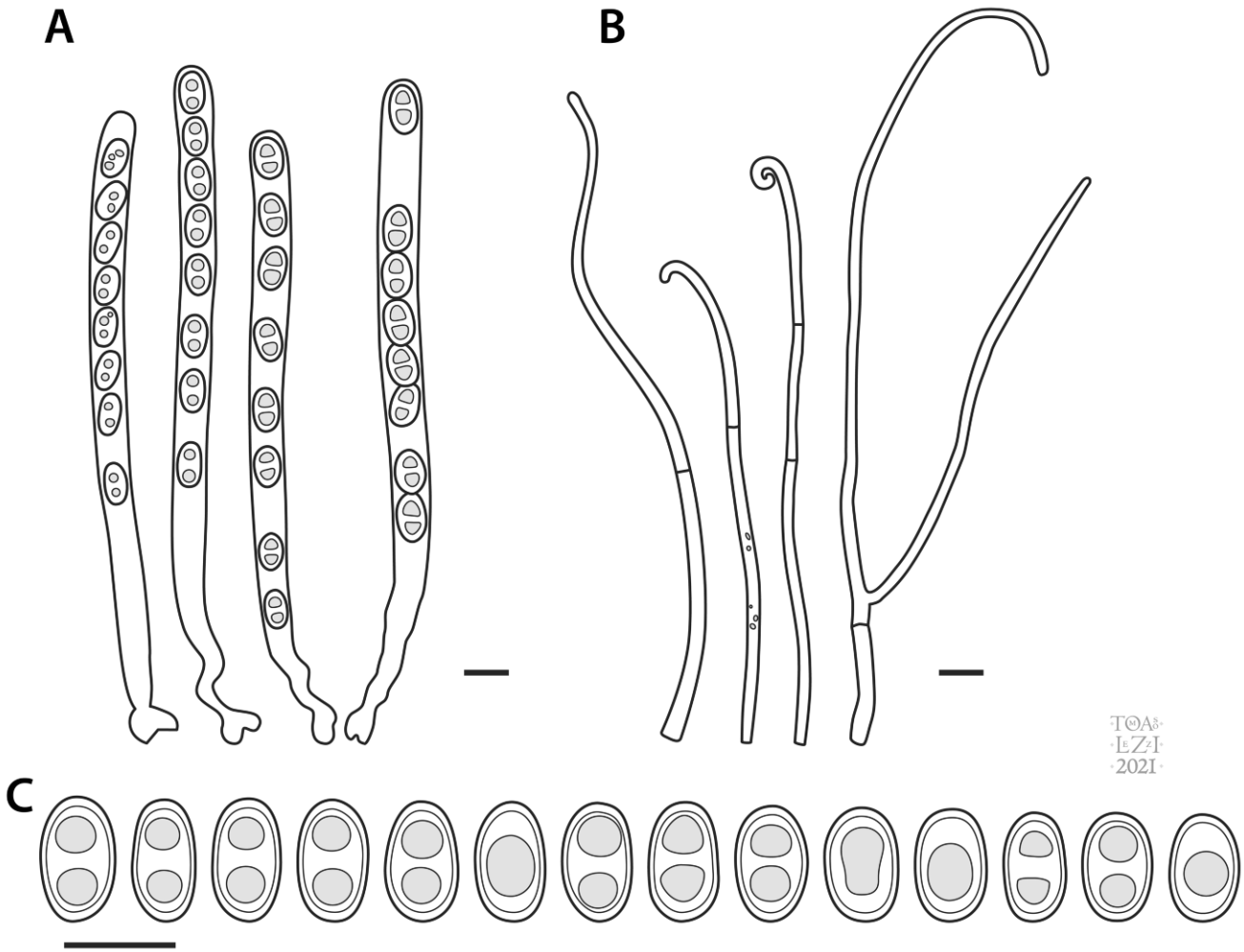

Plate 5 – *Warcupia cupulata*. A: asci with ascospores; B: paraphyses and C: ascospores. Scale bars = 10 µm. Drawing obtained from slides mounted in tap water (T. Lezzi)

Plate 6 – A: *Arpinia luteola* var. *pallidorosea* (GDOR 2950, F. Boccardo). B: *Arpinia inops* (GDOR 1020, F. Boccardo). C: *Acervus epispartius* (TURA 209466, F. Padovan).

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1: M. Carbone – Via Don Luigi Sturzo 173, 16148 Genova, Italy – matteocarb@hotmail.com

2: G. B. Galeotti – Via Iuri Gagarin 2, 06012 Città Di Castello, Italy – gionnys@hotmail.com

3: T. Lezzi – Loc. Forno Vecchio 12, 01021 Torre Alfina, Italy – tomaso@spyrograph.it

4: A. Athanasiadis– Troias 7, 54639 Thessaloniki, Greece – athana31@gmail.com

5: P. Alvarado – ALVALAB, Dr. Fernando Bongera, Severo Ochoa S1.04, 33006 Oviedo, Spain – pablo.alvarado@gmail.com