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Pleurotus opuntiae revisited – An insight to the phylogeny of dimitic *Pleurotus* species with emphasis on the *P. djamor* complex

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ABSTRACT

The name *Pleurotus opuntiae* is indiscriminately used for describing mushrooms with white to off-white to white-grey pilei with short or absent stipe and dimitic hyphal system, which grow on plants of the genera *Opuntia*, *Yucca*, *Agave*, *Phytolacca* etc. However, the outcome of the present study evidences that this name should be reserved for specimens deriving from the Mediterranean area only; an epitype originating from Italy on *Opuntia ficus-indica* is designated. Pertinent material was sequenced by using the internal transcribed spacer region (ITS) and found to be phylogenetically related to *P. djamor* from Kenya and Nigeria, while members of the *P. djamor* complex from other continents were clearly more distant. Results were further corroborated by examining the large subunit of nuclear ribosomal DNA (LSU) and the second subunit of RNA polymerase II (RPB2). The *P. djamor* complex shows high intra-specific polymorphism evidenced by sequence divergence and genetic distance values, presents a cosmopolitan distribution and also comprises material initially identified as *P. flabellatus*, *P. opuntiae*, *P. ostreatoroseus*, *P. parsonsiae* and *P. salmoneostramineus*. An ITS tree including representative specimens from all major *Pleurotus* species is provided for the first time and ambiguous taxa are discussed in the context of new findings.

1. Introduction

The genus *Pleurotus* (Jacq.) P. Kumm. includes several taxa and species-complexes with a world-wide distribution many of which are of high economic interest. However, their study and subsequent exploitation was repeatedly hindered by problems related to ambiguous initial identifications, erroneous use of taxonomic names, and conclusions based on fragmentary and/or not robust data. The advent of DNA sequencing on thoroughly-studied biological material contributed at either elucidating the taxonomic status and phylogenetic relationships within certain groups of *Pleurotus* spp., e.g. *P. cystidiosus* and *P. eryngii* complexes (Zervakis et al., 2001, 2004, 2014) or at documenting the existence/distribution of rare species (e.g. *P. placentodes*, Liu et al., 2016; *P. rickii*, Menolli Jr. et al., 2014). However, a great deal of controversy still exists among other members of the genus.

P. opuntiae (Durieu & Lév.) Sacc. was described on the basis of specimens collected from *Opuntia ficus-indica* (L.) Mill. in Algeria. Giuseppe Inzenga included this species in his Centuria I on Sicilian fungi (1865–1868) after recording it on rotten trunks of *O. ficus-indica* in the farm of Istituto Agrario Castelnuovo in Palermo, Sicily (15 Oct 1866), and of poplar in the Parco della Favorita (Palermo). *P. opuntiae* was further detected on *Opuntia vulgaris* L. in Mallorca (Balearic Islands, Spain) (Barceló y Combis, 1881), as well as on *Opuntia* spp., *Agave americana* L. and *Phytolacca americana* L. in Italy (Sicily and Sardinia), Algeria and Tunisia (Bresadola, 1920, 1928).

More recently, *P. opuntiae* was reported on residues of *O. ficus-indica* in Malta (Briffa and Lanfranco, 1986), on *Ricinus communis* L. in Palermo (Venturella, 1990), on *R. communis* and *Yucca* sp. in Granada (Ortega and Vizoso, 1992), on *Dracaena* sp. in Palermo (Anastase and La Rocca, 1997), on *Phoenix dactylifera* L. in Minorca (Balearic Islands, Spain) (Mir et al., 2008), on *Opuntia* sp. in Cádiz (Spain) (Romera Muñoz and Olivera Amaya, 2013), and in several

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other localities of Italy (Calabria, Sardinia and Sicily) and Spain (pers. comm. with local societies of amateur mycologists). Furthermore, it was also recorded in countries of north – northwest Africa, i.e. in Morocco (Rabat and Tanger) on *A. americana*, *Ficus carica* L., *O. ficus-indica*, *Yucca* sp., *Morus alba* L. and *Pelargonium* sp. (Haimed et al., 2013; Malençon and Bertault, 1975), in Algeria on *Syagrus romanzoffiana* (Cham.) Glassman and *Ricinus* sp. (Malençon and Bertault, 1975), and in Tunisia (Ben Hassine Ben Ali and Stephenson, 2016).

On the other hand, the name *P. opuntiae* was extensively used to describe *Pleurotus* mushrooms occurring in other continents as well. These specimens were also characterized by light colored pilei and dimitic hyphal system, and they were reported to grow on *Espeletia schultzii* Wedd. in Venezuela (Dennis, 1961, 1970), on *Agave* and *Opuntia* spp. in Mexico (Camacho et al., 2012; Montoya et al., 2004), and on *Cordyline australis*, *Araucaria heterophylla* (Salisb.) Franco, *Corynocarpus laevigatus* J.R.Forst. & G.Forst., *Leptospermum scoparium* J.R.Forst. & G.Forst., *Liquidamber styraciflua* L. and *Rhopalostylis baueri* (Hook. f.) H. Wendl. & Drude in New Zealand (Segedin et al., 1995), while few other "*P. opuntiae*" records appeared sporadically in Asia as well (Bao et al., 2004).

The large phenetic plasticity of basidiomes, the overlapping anatomical characters, and the absence of valid sequence data and/or phylogenetic reconstructions for most of the material mentioned above led to significant ambiguities and controversial reports regarding the exact identity and distribution of *P. opuntiae* (Camacho et al., 2012; Hilber, 1982; Petersen and Krisei-Greilhuber, 1999; Segedin et al., 1995). This is rather common among dimitic *Pleurotus* taxa since many of them possess a dubious status and/or affinities to other members of the genus, while significant discrepancies exist regarding pertinent scientific names mentioned in literature and their correspondence/connection to the respective biological entities. This problematic situation is best exemplified by material identified as *P. salmoneostramineus* Lj.N. Vassiljeva, *P. eöus* (Berk.) Sacc., *P. ostreatoroseus* Singer, *P. flabellatus* Sacc., *P. agaves* Dennis, *P. euosmus* (Berk.) Sacc., *P. calyptratus* (Lindblad ex Fr.) Sacc., *P. yuccae* Maire and *P. opuntiae*, all associated to a greater or lesser degree to *P. djamor* (Rumph. ex Fr.) Boedijn (Albertó et al., 2002; Camacho et al., 2012; Guzmán et al., 1993; Nicholl and Petersen, 2000; Petersen, 1995; Vilgalys and Sun, 1994). However, according to the Index Fungorum, the aforementioned names (except of *P. salmoneostramineus*) still maintain a discrete species status, and most of them are referred to as valid/distinct species in numerous publications perpetuating thus the existing confusion.

In the frame of the present study, material originating from the wider topotype area of *P. opuntiae* is examined for the first time by combining morphological characters and sequence data (ITS, LSU and RPB2 markers) for elucidating its taxonomic status and for determining phylogenetic relationships to related species. Particular emphasis is also given at clarifying the use of names associated with several dimitic taxa and at providing an updated phylogeny of selected groups within the genus *Pleurotus*.

2. Methods

2.1. Biological material

Pleurotus opuntiae specimens recorded on *Opuntia ficus indica* (L.) Mill. and *Yucca elephantipes* A. Regel residues in Sicily, Italy, were collected, given the code names SAF 250, SAF 251 and SAF 252, and then subjected to further examination (including DNA sequencing). Pure cultures were established directly from *Pleurotus* basidiomes and were routinely maintained on potato dextrose agar (PDA, Conda). All material (dried specimens and pure cultures) is deposited in the herbarium of the Department of Agricultural, Food and Forest Sciences of the Palermo University (PAL), and/or it is available in pure cultures preserved at the Agricultural University of Athens (Laboratory of General and Agricultural Microbiology).

2.2. Morphology

Morphological characteristics of fresh and dried basidiomes were examined as described elsewhere (Venturella et al., 2015; Zervakis and Balis, 1996; Zervakis et al., 2014), and by using a Zeiss Axioimager A2 Differential Interference Contrast (DIC) microscope at magnifications of up to × 1000. Particular emphasis was placed on microscopic features like basidiospores, basidia, hyphal system and presence of cystidia or cystidia-like elements, which were measured with the aid of an Axiocam camera and the ZEN lite software. In all cases, a minimum of 30 measurements were conducted for each character. Particularly as regards the size range of spores, 5 % of the measurements were excluded from each end of the range and are presented in parentheses, while the quotient (Q) of their dimensions was calculated as the ratio of spore length over spore width. Me and Qe represent the median values of length/width and quotient respectively. Color terminology used in morphological descriptions follows Ridgway (1912).

2.3. DNA extraction, PCR amplification, sequencing and data assembly

DNA was obtained from either dried specimens or from mycelium which was harvested by scraping-off the surface of cultures grown on PDA in Petri dishes. Samples (approximately 0.2 g) were pulverized by using a micropestle in the presence of sterile sand and liquid nitrogen. Total DNA was subsequently extracted through the use of microcentrifuge tubes (NucleoSpin Plant II DNA Extraction Miniprep System; Macherey and Nagel, Germany) by following the manufacturer's protocol with minor modifications. DNA was quantified by using a Nanodrop ND-1000 spectrometer (Nanodrop Technologies, USA) and its concentration was adjusted at 50 ng μl^{-1} for the PCR reaction. Aliquots were stored at −20 °C.

Phylogenetic relationships among *Pleurotus* species were investigated through the analysis of the internal transcribed spacer region (ITS) and the large subunit (LSU) of nuclear ribosomal RNA gene, and the second subunit of RNA polymerase II (RPB2). PCR amplification was conducted using the pairs of primers ITS-1F/ITS4b or ITS1/ITS4 for ITS (Gardes and Bruns, 1993; White et al., 1990), LR0R/LR5 for LSU (domains D1-D5; Cubeta et al., 1991), and fRPB2-5F/bRPB2-7R2 for RPB2 (domains 5 to 7; Matheny et al., 2005). PCR reactions were prepared from 50 ng DNA in 50 μl PCR reagent containing 1.5 U Takara HiFi polymerase (High Fidelity PCR system, Takara Bio USA, Inc.), 2 mM of each dNTP and 0.25 μM of each PCR primer. The amplification reactions were conducted on a DNA Engine Thermal Cycler (Bio-Rad, USA) and the conditions were as follows: an initial denaturation at 95 °C for 3 min, followed by 35 cycles of denaturation at 95 °C for 30 s, annealing at 50 °C for ITS1/ITS4 and LR0R/LR5 or 52 °C for ITS-1F/ITS4b and fRPB2-5F/bRPB2-7R2 for 30 sec, and extension at 72 °C for 1 min for ITS and 2 min for LSU and RPB2. A final synthesis at 72 °C for 5 min was included to complete the reaction.

PCR products were purified by microcentrifugation using PureLink PCR purification kit (Invitrogen, USA), and were then processed for bidirectional Sanger sequencing at CEMIA (University of Thessaly, Greece; http://cemia.eu/). The resulting chromatograms were proofread (viewed and manually edited) and the contigs were assembled in MEGA X (Kumar et al., 2018). *P. opuntiae* sequences were deposited in GenBank under the accession numbers MH620770 to MH620772 for ITS (Table 1), MK182779 to

Table 1
Biological material examined in this study by morphological criteria and rDNA ITS-5.8S sequencing: identity of each *Pleurotus* specimen (accompanied with original determination when different; placed in brackets), geographic origin, voucher/specimen code, GenBank accession number, and pertinent reference (as it appears in GenBank). Relevant information is also provided for all other sequences used in the present work.

Taxon identity (original determination)	Geographic Origin	Voucher/Specimen Code	GenBank Assession No.	Reference
P. abieticola	Russia	6554	AY450348	Petersen and Hughes, 1997
P. abieticola	China, Tibet	HKAS45720	KP771696	Yang et al., unpublished
P. agaves	Mexico, Tlaxcala	CP-194	GU722262	Huerta et al. (2010)
P. agaves	Mexico, Cuernavaca	ECS-0165	GU722264	Huerta et al. (2010)
P. agaves (“*P. opuntiae*”)	Mexico	ET3313	AY450339	Hughes and Petersen, unpublished
P. agaves (“*P. opuntiae*”)	Mexico	TENN52368	AY450340	Hughes and Petersen, unpublished
P. australis	Australia	VT1953	AY315758	Zervakis et al. (2004)
P. australis	New Zealand	PDD59215	AY315761	Zervakis et al. (2004)
P. calyptratus	Austria	9065, TENN57451	AY450338	Hughes and Petersen, unpublished
P. calyptratus	Slovakia	CBS 325.85	EU424283	Gao et al., unpublished
P. calyptratus	Russia	1935	KF932720	Shnyreva and Shnyreva (2015)
P. calyptratus	South Korea	P71	KY962487	Yan et al., unpublished
P. citrinopileatus	South Korea	P68	KY962484	Yan et al., unpublished
P. citrinopileatus	China	YAASM1585	KX836372	Li et al. (2017)
P. cornucopiae	unknown	WC608	AF079582	Thon and Royse (1999)
P. cornucopiae	Austria	8763, TENN55191	AY450341	Hughes and Petersen, unpublished
P. cornucopiae	South Korea	P69	KY962485	Yan et al., unpublished
P. cystidiosus	USA	D419	AY315774	Zervakis et al. (2004)
P. djamor	Papua New Guinea	CBS 100134	EU424287	Gao et al., unpublished
P. djamor	Cuba	CBS596.96	FJ040176	Gao, unpublished
P. djamor	Mexico, Talquian	ECS-0123	GU722265	Huerta et al. (2010)
P. djamor	Mexico, Cuernavaca	ECS-0159	GU722273	Huerta et al. (2010)
P. djamor	India	CBE 12	JX625130	Thiribhuvanamala et al., unpublished
P. djamor	Brazil	unknown	KF280325	Menolli Jr. et al. (2014)
P. djamor	Brazil	SP445805	KF280327	Menolli Jr. et al. (2014)
P. djamor	Brazil	SP445789	KF280329	Menolli Jr. et al. (2014)
P. djamor	Kenya	KKF8428	KJ754106	Otieno et al. (2015)
P. djamor	Kenya	KKF2947	KJ754107	Otieno et al. (2015)
P. djamor	Kenya	KKF2710	KJ754108	Otieno et al. (2015)
P. djamor	Kenya	KKF0121	KJ754109	Otieno et al. (2015)
P. djamor	Peru	IB36	KJ831854	Gazis et al., unpublished
P. djamor	Australia, NR	MEL:2382775	KP012957	Bonito et al., unpublished
P. djamor	Dominican Republic	TENN F-59778	KP026246	Hughes et al., unpublished
P. djamor	Nigeria, Elebele	ELEB27	KT273359	Adedokun et al. (2016)
P. djamor	India	IPL/MC/PD-1	KT768094	Chakraborty et al., unpublished
P. djamor	Vietnam, Cat Tien	LE-BIN 3279	KY328722	Psurtseva, unpublished
P. djamor	Malaysia	FUM-093	KY951475	Avin et al. (2017)
P. djamor	Pakistan	1092014J	KX056435	Razzaq and Niazi, unpublished
P. djamor	Mexico	CC050	KX573921	Aguilar Doroteo et al., 2018
P. djamor	Mexico, Pue	CC052	KX573923	Aguilar Doroteo et al., 2018
P. djamor	Mexico	CC054	KX573925	Aguilar Doroteo et al., 2018
P. djamor	Mexico	CC056	KX573927	Aguilar Doroteo et al., 2018
P. djamor	China	HKAS90179	KX836373	Li et al. (2017)
P. djamor (“*P. agaves*”)	Mexico, Coatepec	ECS-0176	GU722275	Huerta et al. (2010)
P. djamor (“*P. djamor* var. *roseus*”)	unknown	ABM1049203	KC582639	Habuan Hanipah, unpublished
P. djamor (“*P. djamor* var. *roseus*”)	unknown	ABM1049204	KC582640	Habuan Hanipah, unpublished
P. djamor (“*P. flabellatus*”)	Malaysia	ATCC38137	AY265827	Choi et al., unpublished
P. djamor (“*P. flabellatus*”)	unknown	ATCC38140	AY368660	Choi et al., unpublished
P. djamor (“*P. flabellatus*”)	unknown	ACCC51447	EU424303	Gao et al., unpublished
P. djamor (“*P. flabellatus*”)	India, Pechiparai	P7	KT970056	Arul Kumar and Muthumary, unpublished
P. djamor (“*P. opuntiae*”)	India	SR624-PO	KY214255	Krishnapriya et al. (2017)
P. djamor (“*P. opuntiae*”)	South Korea	P101	MG282441	Yan, unpublished
P. djamor (“*P. opuntiae*”)[a]	New Zealand	ICMP 11566	MH395961	Weir et al., unpublished [PDD:58730]
P. djamor (“*P. opuntiae*”)[a]	New Zealand	ICMP 11670	MH395966	Weir et al., unpublished [PDD:48658]
P. djamor (“*P. opuntiae*”)[a]	New Zealand	ICMP 11671	MH395967	Weir et al., unpublished [PDD:48644]
P. djamor (“*P. ostreatoroseus*”)	South Korea	P94	MG282434	Yan, unpublished
P. djamor (“*P. parsonsiae*”)	New Zealand	ICMP 18169	MH395975	Weir et al., unpublished [PDD:101089]
P. djamor (“*P. salmoneostramineus*”)	South Korea	ASI 2104	AY265844	Choi et al., unpublished
P. djamor (“*P. salmoneostramineus*”)	South Korea	ASI 2172	AY265845	Choi et al., unpublished
P. djamor (“*P. salmoneostramineus*”)	China	Yuanlin No. 1	AY728273	Lin et al., unpublished
P. djamor (“*P. salmoneostramineus*”)	unknown	ACCC50836	EU424302	Gao et al., unpublished
P. dryinus	Denmark	7947	AY450343	Hughes and Petersen, unpublished
P. dryinus	Czech Republic	CBS 44977	EU424291	Gao et al., unpublished
P. dryinus	The Netherlands	CBS 72483	EU424293	Gao et al., unpublished
P. eous	India	SR624-PE	KY214257	Krishnapriya et al. (2017)
P. eous	South Korea	P109	MG282448	Yan, unpublished
P. eryngii	Greece	LGAMP63	HM998811	Zervakis et al. (2014)
P. eryngii	Italy	UP10	HM998817	Zervakis et al. (2014)
P. euosmus	United Kingdom	CCRC36212	AY265826	Choi et al., unpublished
P. euosmus	United Kingdom	CBS 307.29	EU424298	Gao et al., unpublished
P. fuscosquamulosus	Greece	LGAMP50	AY315789	Zervakis et al. (2004)

Table 1 (*continued*)

Taxon identity (original determination)	Geographic Origin	Voucher/Specimen Code	GenBank Assession No.	Reference
P. giganteus	Australia, NT	MEL:2382605	KP012913	Bonito et al., unpublished
P. giganteus	China, Yunnan	MFLU14-0638	KP135560	Karunarathna et al., 2016
P. giganteus (“*Panus giganteus*”)	China	LS	HM245780	Dong and Li, unpublished
P. giganteus (“*Panus giganteus*”)	China	ZZ	HM245788	Dong and Li, unpublished
P. levis	unknown	TENN 58298	AF345662	Alberto et al., unpublished
P. levis	Mexico, Puebla	IE-771	KC894735	Gonzalez-Tijera et al., unpublished
P. levis	USA, Texas	DPL6135	KP026244	Hughes et al., unpublished
P. levis (“*P. dryinus*”)	unknown	ASI2123	AY265823	Choi et al., unpublished
P. levis (“*P. dryinus*”)	USA	FLAS-F-61321	MH211881	Kaminsky et al., unpublished
P. nebrodensis	Italy	UPA28	HM998818	Zervakis et al. (2014)
P. nebrodensis	Greece	HIK125	HM998826	Zervakis et al. (2014)
P. opuntiae	Italy, Palermo	SAF 250	MH620770	This study
P. opuntiae	Italy, Catania	SAF 251	MH620771	This study
P. opuntiae	Italy, Palermo	SAF 252	MH620772	This study
P. ostreatus	France	CBS 291.47	EU424309	Gao et al., unpublished
P. ostreatus	Italy, Trentino	CBS 375.51	EU424310	Gao et al., unpublished
P. placentodes	China	HKAS51745	KR827693	Liu et al. (2016)
P. placentodes	China, Yunnan	HKAS57781	KR827694	Liu et al. (2016)
P. populinus	USA	ATCC 90083	AY368667	Choi et al., unpublished
P. populinus	USA, New Mexico	TENN F-59144	KP026249	Hughes et al., unpublished
P. pulmonarius	USA	TENN F-60548	KP026250	Hughes et al., unpublished
P. pulmonarius	Russia	TENN F-59627	KP026253	Hughes et al., unpublished
P. pulmonarius (“*P. opuntiae*”)	India, Garhwal	ATCC90202	AY265838	Choi et al., unpublished
P. pulmonarius (“*P. opuntiae*”)	South Korea	P124	MG282463	Yan, unpublished
P. pulmonarius (“*P. opuntiae*”)	South Korea	P132	MG282471	Yan, unpublished
P. pulmonarius (“*P. eous*”)	South Korea	P139	MG282478	Yan, unpublished
P. pulmonarius (“*P. eous*”)	South Korea	P149	MG282488	Yan, unpublished
P. purpureo-olivaceus	New Zealand	PDD 91612	GQ411512	Fukami et al., 2010
P. purpureo-olivaceus	New Zealand	PDD:91632	GQ411523	Fukami et al., 2010
P. rickii	Brazil	SP445788	KF280341	Menolli Jr. et al. (2014)
P. rickii	Brazil	SP445787	KF280342	Menolli Jr. et al. (2014)
P. tuber-regium	Australia	RV95/175.1	AF109965	Isikhuemhen et al. (2000)
P. tuber-regium	Ghana	PTR3	AF109977	Isikhuemhen et al. (2000)
P. tuber-regium	Nigeria	PtW1	AF109979	Isikhuemhen et al. (2000)
P. tuber-regium	China	130433	KM405793	Liu et al., unpublished

[a] Deposited to GenBank under the name “*P. parsonsiae*” but the material sequenced corresponds to “*P. opuntiae*” (Segedin et al., 1995).

MK182781 for LSU (Supplementary Table 1), and MK189435, MK189436 and MK239648 for RPB2 (Supplementary Table 2).

2.4. Sequence alignment and phylogenetic analyses

In addition to the biological material examined in this study, 103 additional ITS1-5.8S-ITS2 *Pleurotus* sequences representing 28 names (Table 1), 22 LSU sequences representing 10 names (Supplementary Table 1) and 13 RPB2 sequences representing five names (Supplementary Table 2) were retrieved from the GenBank and included in the analysis. Selection was made on the basis of the extra information they could confer especially as regards the highest possible representation of taxa associated with the *P. djamor* complex.

Sequences were aligned by using the online version of MAFFT v.7 (Katoh and Standley, 2013; https://mafft.cbrc.jp/alignment/software/) by applying the interactive refinement method Q-INS-I for ITS region and L-INS-I for the LSU and RPB2 datasets, and then alignments were manually optimized by MEGA X (Kumar et al., 2018).

Phylogenetic analyses were based on maximum likelihood (ML) and Bayesian inference (BI). Bayesian analyses were performed in MrBayes v3.2.1 software (Ronquist et al., 2012) using the optimal model of nucleotide substitution according to the Akaike Information Criterion (AIC) for each dataset as implemented by TOPALi v2.5 (Milne et al., 2008); GTR + G was used for ITS, GTR + G + I for LSU and SYM + G for RPB2. For estimation of posterior probabilities (PP), two parallel searches on four chains sampled every 100 generations were run from a random starting tree for a total of 5,000,000 Markov chain Monte Carlo (MCMC) simulation generations. Potential scale reduction factors (PSRF) were set to 1.0 for all parameters. The effective sampling size (ESS) values were over 200 and the average standard deviation of split frequencies was less than 0.01. Subsequently, for each dataset, the sampled trees were summarized and a majority rule consensus tree was built after omitting the first 25 % of trees as burn-in. The ML analyses were conducted with RAxML v. 8 (Stamatakis, 2014), and assessed through the CIPRES Science Gateway-web portal (Miller, 2010; http://www.phylo.org/) by employing the GTR + GAMMA substitution model. ML bootstrap (BS) was computed with a rapid analysis of 1000 pseudoreplicates to assess branch confidence and search for the best-scoring ML tree. Significance thresholds were set >70 % for bootstrap (BP) and >95 % for posterior probability (PP). Trees were visualised using iTOL v.4.3.2 (Letunic and Bork, 2016). *Hohenbuehelia auriscalpium* (EF409725) and *H. mastrucata* (EF409737) were used as outgroups in the ITS tree, while monomitic *Pleurotus* spp. served as outgroups in LSU and RPB2 trees.

Evolutionary divergence (p-distance) within and between selected *Pleurotus* taxa was calculated by MEGA X software (Kumar et al., 2018) as the proportion (p) of nucleotide sites at which two sequences being compared are different, and it is obtained by dividing the number of nucleotide differences by the total number of nucleotides compared. Moreover, percent ITS sequence identity was estimated by using ClustalOmega (Sievers and Higgins, 2018) through the EMBL-EBI portal, and were then visualized by box–plot diagrams.

3. Results and discussion

The bad condition of the holotype (Camacho et al., 2012; Petersen and Krisei-Greilhuber, 1999), the absence of any pertinent specimens recently collected from the topotype area (Algeria) and the need to process/study authoritative material of *P. opuntiae* led us to designate an epitype. This particular specimen was isolated in a locality not far from where *P. opuntiae* was initially described and from the same host/substrate on which it was originally recorded.

Pleurotus opuntiae (Durieu & Lév.) Sacc., Sylloge Fungorum 5, 363 (1887).

≡ *Agaricus opuntiae* Durieu & Lév., Exploration scientifique de l'Algérie 1–5, t. 32:1 (1846)
≡ *Dendrosarcus opuntiae* (Durieu & Lév.) Kuntze, Revisio generum plantarum 3(2), 464 (1898)
≡ *Panellus opuntiae* (Durieu & Lév.) Z.S. Bi, Acta Mycol. Sin., 286 (1986)
≡ *Pleurotus ostreatus* subsp. *opuntiae* (Durieu & Lév.) A. Ortega & Vizoso, Documents Mycologiques 22(86), 35 (1992).

Holotype: ALGERIA, as *A. opuntiae* Durieu & Lév., Exploration scientifique de l'Algérie (1846) 15, pl. 32, Fig. 1 (PC!); type specimen outside label stating "sur … vieux tronc pourri de Cactus opuntia", 22 Jan 1840 (see also Petersen and Krisei-Greilhuber, 1999).

Epitype (designated here): ITALY, Campofelice di Roccella (province of Palermo, north Sicily), 10 m a.s.l., 37°59'21"N – 13°53'24"E, in a cultivated flowerbed of a resort house on rotten roots of *Opuntia ficus indica* (L.) Mill. at the edge of an irrigated citrus grove, 15 May 2018, coll. G. Domina, herbarium code SAF252, culture collection accession number 06SAF.

3.1. Morphological description (Figs. 1 and 2)

Basidiomes gregarious, caespitose or imbricate. Pileus 5–10 (–30 cm), conchate, reniform or flabelliform, initially convex to finally applanate to concave, margin slightly involute; whitish or grayish ("*10 gray" to "pallid neutral gray" to "pale neutral gray"), then cream ("light buff") or beige ("pale pinkish buff"), radially appressed fibrillose to squamulose; cuticle partially separable. Lamellae deeply decurrent, corrugated, with lamellulae anastomosing near the stem, white then cream ("light buff") to yellowish-cream ("ivory yellow"), crowded, width 1–2 mm. Stipe absent, or if present rudimentary, short, eccentric or lateral, whitish, then cream ("light buff") or beige ("pale pinkish buff"). Flesh tender then fibrous, white; smell faint, fungoid; taste mild. Spore-print white.

Hyphal system dimitic; generative hyphae 3–6.5 (-9.5) μm diam., hyaline, thin to slightly thick-walled (up to 1 μm thick), with numerous and prominent clamps, moderately branched, tightly interwoven in the trama of pileus and stipe, rather loosely interwoven in lamellae trama; skeletal hyphae 3–5 μm diam. aseptate, rarely and poorly branched, hyaline to yellowish in KOH, thick-walled (up to 2.5 μm), solid, progressively (with age) dominating in the trama of pileus and stipe, usually present in lamellae trama (but may be absent in younger basidiomes). Lamellae trama irregular. Basidiospores (n = 80/2) (8.7-)9.2–10.8 (-12.0) × (4.2-)4.3–4.9 (-5) μm, Q = (1.8-) 2–2.4 (-2.5) (Me = 10.1 × 4.6 μm, Qe = 2.2), oblong-ellipsoid to subcylindrical, hyaline, thin-walled, with numerous oil drops, non-amyloid or dextrinoid, hillar appendage lateral, short and indistinct, with shallow suprahillar depression. Basidia 25-42 × 6–8 μm, slenderly clavate to subcylindrical, clamped; sterigmata 4, up to 8.5 μm long. Pleurocystidia absent; however, slightly projecting, fusoid basidioles observed. Lamellae edge sterile, cheilocystidia (pseudocystidia) arising from tramal generative hyphae, 19-44 × 6–9.5 μm, in clusters, very variable in shape, more often subcylindrical to subclavate, otherwise obovate to utriform or pyriform, and then up to 19 μm broad, or polymorphic, bended, rostrate with digitiform outgrowths; hyaline, thin-walled to slightly thick-walled, clamped at base. Pileipellis a thin cutis of periclinal hyphae, with cylindrical apices 3–7 μm diam., at disc partly ascending and tortuous, clamped.

Additional material examined (apart of the epitype): (i) on the trunk of a living plant of *Y. elephantipes* A. Regel, 24 Oct 2016, Garden of the Department of Agricultural, Food and Forest Sciences, University of Palermo (Sicily, Italy), 14 m a.s.l., G. Venturella, code SAF250 (ii) on rotten roots of *O. ficus-indica* (L.); Mill., 11 Apr 2016, Ficod'india Caudarella Farm, at the vicinity of S. Pietro wood,

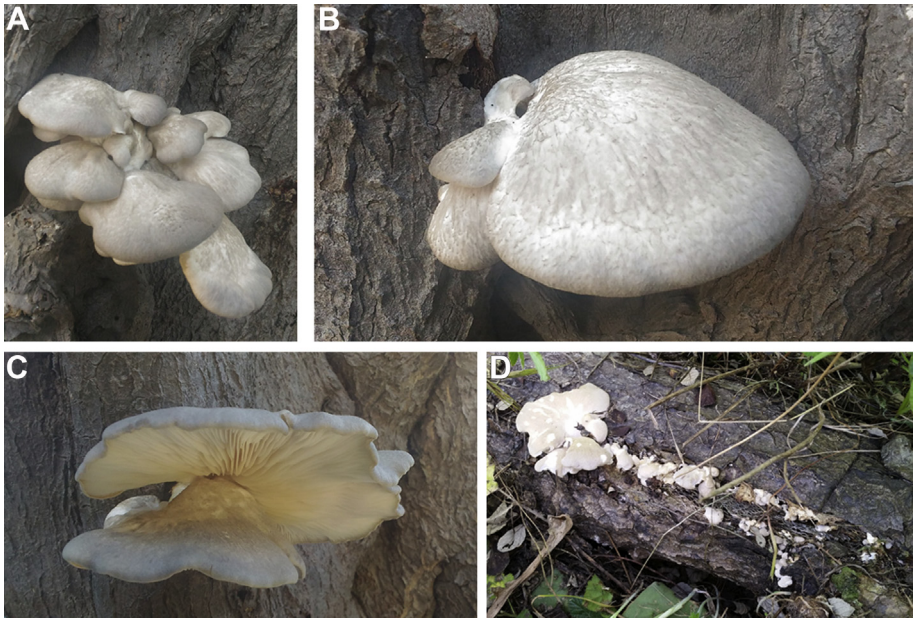

Fig. 1. (A), (B) and (C) basidiomes of *Pleurotus opuntiae* fruiting in autumn on *Yucca elephantipes* living trees; (D) basidiomes of *P. opuntiae* growing in spring on rotten cladodes of *Opuntia ficus-indica*.

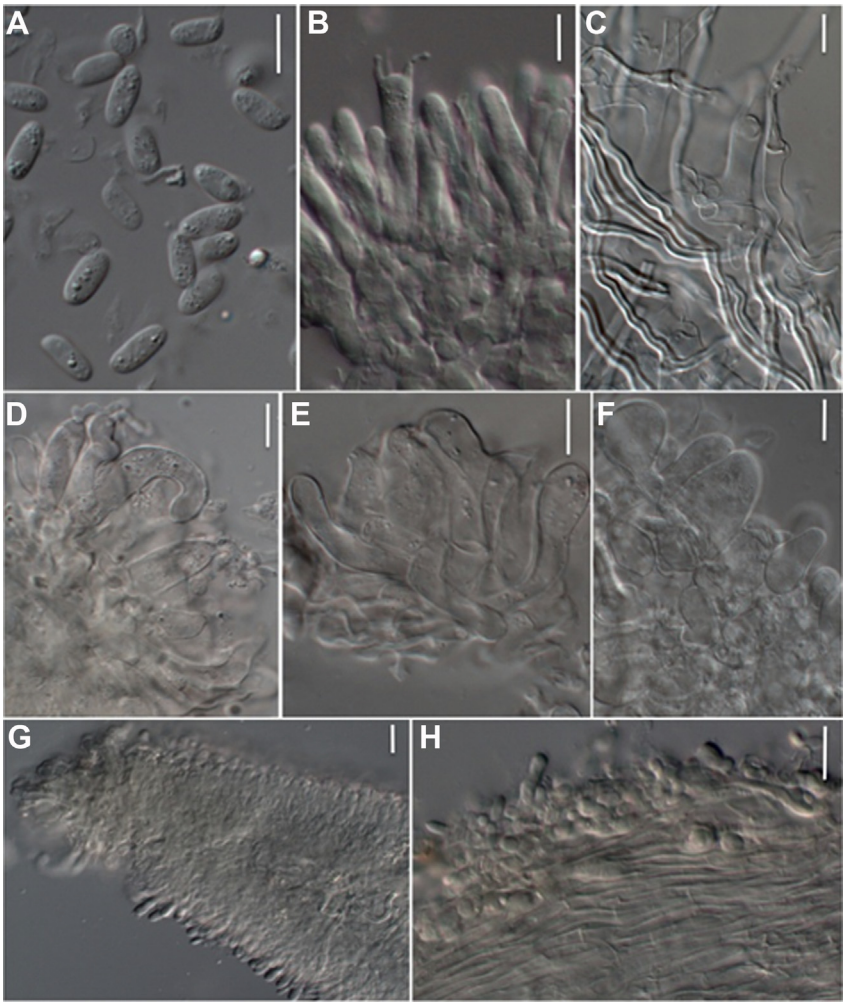

Fig. 2. (A) basidiospores; (B) basidia and basidioles; (C) generative and skeletal hyphae from lamella-trama; (D) to (F) cheilocystidia; (G) lamella section; (H) pileipellis. Bar = 10 μm (A–F); bar = 20 μm (G–H).

Caltagirone (province of Catania, eastern Sicily, Italy), 390 m a.s.l., P. Inglese, material's code number SAF251.

P. opuntiae specimens (SAF 251 and SAF 252) are reported for the first time from commercial cactus pear (*O. ficus-indica*) orchards which are cultivated for fruit production according to the principles of permaculture. Hence, manure is used instead of chemical fertilizers, no pesticides are used for crop management, while prunings (cladodes removed during spring and summer) are chopped off and left onto the soil along each plant-row. Such cultivation practices differ substantially from those commonly adopted in cactus pear intensive production in Sicily usually supported by synthetic inputs.

It is also worth noting that *Y. elephantipes* as well as plants of the genus *Opuntia* are native to the Americas. In particular, *O. ficus-indica* seems to derive from central Mexico (Griffith, 2004), it was probably introduced in Spain after the first or second trip of Columbus to the new continent, and it soon spread throughout the Mediterranean area becoming a characteristic element of its landscape and a crop commodity in several countries (Inglese et al., 2017). On the other hand, *Y. elephantipes* was introduced in Sicily in the early 19th century, and it is widely cultivated in private and public gardens.

Although non-monomitic genera are rare in Agaricales (Corner, 1981; Pegler, 1983; Singer, 1986), 'dimitic' hyphal constructions (as it was the term used by Albertó et al., 2002) or imperfect skeletal hyphae do appear in the genus *Pleurotus* and their use was of primary importance at accommodating taxa into different sections, i.e. *Lepiotarii* (Fr.) Pilát, *Calyptrati* Sing., *Pleurotus*, *Lentodiellum* (Murr.) Sing. and *Tuberregium* Sing. sensu Singer (1986), or subgenera, i.e. *Pleurotus* and *Lentodiopsis* (Bubák) O. Hilber sensu Hilber (1982). However, only scarce and fragmentary information exists regarding the phylogenetic position of a few dimitic *Pleurotus* species, while no single study has ever examined ITS sequences representing all dimitic taxa and their phylogenetic relationships.

ITS amplification resulted in sequences of 581–655 bp. The topologies in the respective phylogenetic trees generated from ML and BI analyses were almost identical. On the ML tree selected to represent the phylogeny within the genus *Pleurotus* (Fig. 3), *P. purpureo-olivaceus* as well as *P. dryinus* and *P. tuber-regium* (the latter two are dimitic) appear at basal positions. A distinct clade was further formed by members of the subgenus *Coremiopleurotus* Hilber, i.e. *P. australis*, *P. cystidiosus* and *P. fuscosquamulosus* in accordance to previous findings (Zervakis et al., 2004). The rest of the monomitic species formed a well-supported clade (BS: 100 % and BPP: 1.00) which was sister to one consisting of dimitic taxa only (96 % and 1.00).

As regards the former group, it included *P. placentodes*, *P. abieticola*, *P. pulmonarius*, *P. ostreatus*, *P. nebrodensis*, *P. eryngii* and *P. populinus*. Of interest was that five ITS sequences deposited under the names *P. opuntiae* (India and South Korea) and *P. eous* (South

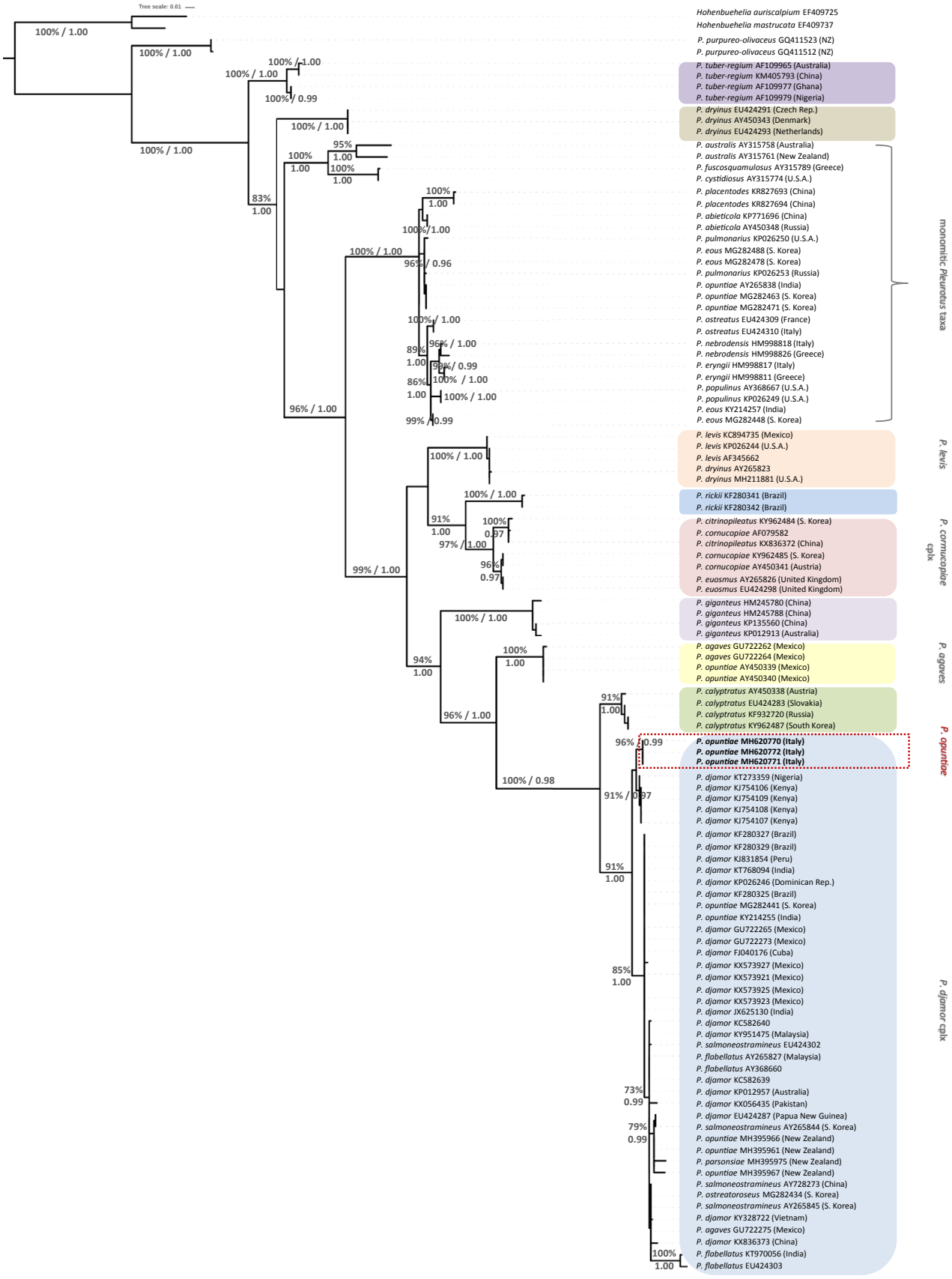

Fig. 3. Phylogenetic tree of *Pleurotus* species inferred from maximum likelihood (ML) analysis based on ITS sequence data. ML bootstrap values (BS) ≥ 70 % and Bayesian posterior probabilities (BPP) ≥ 0.95 are shown above and below the branches (or before and after the slash), respectively. Sequences generated in this study are indicated in boldface, while sequences presented on coloured background correspond to dimitic taxa. Names appearing on the far-right part represent the correct species identity.

Korea) clustered together with *P. pulmonarius* indicating misidentifications in the respective material (Table 1). Indeed, one of the "P. opuntiae" sequences (AY265838, ATCC 90202) derived from material isolated from India was previously reported to be intercompatible with *P. pulmonarius* (Bao et al., 2004). However, two other *P. eous* sequences (KY214257 and MG282448 from India and South Korea respectively) were clearly separated from other monomitic taxa (99 % and 0.99) evidencing thus their distinct taxonomic status at species level. It is noteworthy that in MycoBank, *P. eous* (orthographic variant of *P. eöus*) is considered as a synonym of *P. djamor*. This is apparently a mistake since no sequences under the name *P. eous* were associated with any dimitic *Pleurotus* examined in this study. The problem could have been caused due to the close morphological resemblance of *P. eous* (also called "pink oyster mushroom" occurring in the subtropics; Rajarathnam and Bano, 1987; Singh and Rajarathnam, 1977) to *P. djamor* (Corner, 1981; Pegler, 1972).

Another large, well-supported cluster (99 % and 1.00) consisted entirely of dimitic taxa by including *P. levis*, *P. rickii*, *P. cornucopiae* complex, *P. giganteus*, *P. agaves*, *P. calyptratus* and the *P. djamor* complex (Fig. 3). The last formed a terminal subclade of high support (91 % and 1.00) which was further divided in two sister groups. One of them (85 % and 1.00; corresponding to the "core part" of *P. djamor*) included sequences from material with a cosmopolitan distribution (excluding Europe and Africa), e.g. Brazil, Peru, Cuba, Dominican Republic, Mexico, China, India, Malaysia, Pakistan, South Korea, Vietnam, Papua New Guinea, Australia and New Zealand. It is noteworthy that several ITS sequences placed within this group were labeled with other names, i.e. *P. agaves* (GU722275), *P. flabellatus* (AY265827, AY368660, KT970056, EU424303), *P. opuntiae* (KY214255, MG282441, MH395961, MH395966, MH395967), *P. ostreatoroseus* (MG282434), *P. parsonsiae* (MH395975) and *P. salmoneostramineus* (AY265844, AY265845, AY728273, EU424302). While the conspecificity/synonymy of *P. flabellatus*, *P. ostreatoroseus* and *P. salmoneostramineus* to *P. djamor* was indicated in the past mainly through morphological and/or mating studies (Corner, 1981; Nicholl and Petersen, 2000; Petersen, 1995), very limited information exists on the application of molecular approaches to confirm such conclusions. In addition, it was shown for the first time that material under the name "P. opuntiae" from Asia and New Zealand belongs also to *P. djamor*. Especially as regards sequences from New Zealand's specimens recently deposited in GenBank under the name "P. parsonsiae" by Weir et al. (unpublished data), they either derive from material initially identified as "P. opuntiae" (i.e. culture collection code ICMP 11566, accession no. MH395961; ICMP 11670, MH395966; ICMP 11671, MH395967; culture collection codes were provided by Segedin et al., 1995) or from specimens wrongly identified as "P. parsonsiae" since the morphological description of the latter taxon (e.g. basidiomes with soft consistency and monomitic trama, few sclerified generative hyphae and abundant oleiferous hyphae; Segedin et al., 1995) does not correspond to *P. djamor*. The extent of erroneous misidentifications is indicative of the difficulty in assessing material of this group on the basis on morphological criteria only.

The high ITS heterogeneity presented by *P. djamor* specimens is hereby documented by the increased evolutionary divergence (p-distance) detected within this group, i.e. average value: 0.44 % for the "core part" (38 sequences, max. value: 1.87 %) or 0.64 % for the complex excluding *P. opuntiae* but including material labelled as *P. djamor* from Africa (43 sequences, max. value: 2.34 %). These average values are significantly higher than the respective intraspecific values reported for various species of the genera *Entoloma* or *Craterellus* (e.g. < 0.30 %; Matheny et al., 2010; Kondo et al., 2017), while the maximum values quoted for *P. djamor* fall within the lower range of interspecific values found in pairwise comparisons between different members of these genera (e.g. 1.60–3.10 %). Such variability is further evidenced by the low percentages of sequence identity within *P. djamor* (average values: 95.05 % when 38 sequences were taken into account, and 94.19 % when material from Africa was also considered; Fig. 4). In contrast, intraspecific sequence identity ranges from 96.63 % (*P. giganteus*) to 100.00 % (*P. dryinus*) for the rest of the dimitic taxa examined in this work, which however included a lower number of sequences and/or were of less diverse geographic origin.

The sister cluster to the "core part" of *P. djamor* was composed of two well supported groups, one was formed by five sequences (91 % and 0.97) from material identified as *P. djamor* which was isolated in Kenya and Nigeria (Adedokun et al., 2016; Otieno et al., 2015), while the other terminal group (96 % and 0.99) included three sequences from Italian (Sicilian) specimens examined in the frame of our study. According to the data presented herein, the latter corresponds to *P. opuntiae* which holds a distinct phylogenetic position within the *P. djamor* complex; it shows relatively high values of ITS sequence identity in pairwise comparisons (average: 96.49 %, max: 97.42 %) versus African specimens only, and rather low (average: 91.41 %, max: 95.06 %) versus *P. djamor* material of more distant origins (Fig. 4). Similarly, specimens from Africa (Kenya and Nigeria) originally identified as *P. djamor* present low affinity when compared to the "core part" of this complex (average: 90.74 %, max: 93.99 %), and therefore they deserve further consideration prior to their final assessment.

To further examine/confirm the phylogenetic position of *P. opuntiae* within the *P. djamor* complex, two additional markers were used (LSU and RPB2). Their amplification resulted in sequences of 935–944 bp and 910-949 bp, respectively. As it was the case with ITS, topologies in the resulting trees generated from ML and BI analyses were almost identical, and the ML trees were selected to represent the relationships among the material studied. Both LSU and RPB2 phylogenies evidenced the distinct grouping of the three *P. opuntiae* specimens (of Italian origin) in respect to material deposited as *P. djamor* (Fig. 5A,B, respectively). Especially as regards LSU, nine *P. djamor* sequences (including three GenBank entries under the names *P. flabellatus* and *P. salmoneostramineus*; Fig. 5A) formed a sister group (100 % and 1.00) with *P. opuntiae*; the latter corresponded to a terminal well-supported subclade (100 % and 1.00; Fig. 5A). When RPB2 sequences were examined, an identical (to the previous one) topology was obtained for *P. djamor* and *P. opuntiae*, which clustered in two separate -albeit closely related- groups with high support (95 % and 1.00 for *P. opuntiae*; Fig. 5B).

In their study on *Pleurotus* type specimens, Petersen and Krisai-Greilhuber (1999) referred to three concepts related to the use of the name *P. opuntiae*: the first corresponded to the Algerian specimen serving as type of this species (Durieu and Léveillé, 1846), the second to fleshy basidiomes with prominent stipe appearing on *Agave* plants in Mexico (Petersen, 1995) and the third to New Zealand material growing on various substrates (Segedin et al., 1995). As regards the last one, mating tests versus *P. djamor* evidenced that they were intercompatible (Petersen, 1995), an outcome which is supported by molecular data of the present work as previously explained. Then, the type specimen was morphologically compared to Mexican "P. opuntiae", and Petersen and Krisai-Greilhuber (1999) arrived at the conclusion that "… *P. opuntiae* fruits in North Africa and Mexico, and that the Mexican use of the name is correct". However, in the frame of our study, one ITS sequence from the same Mexican material (TENN 52368, identified as "P. opuntiae") was examined and it was demonstrated that it belongs to *P. agaves*. The latter is a distinct phylogenetic species as it was previously reported (Vilgalys et al., 1996; Huerta

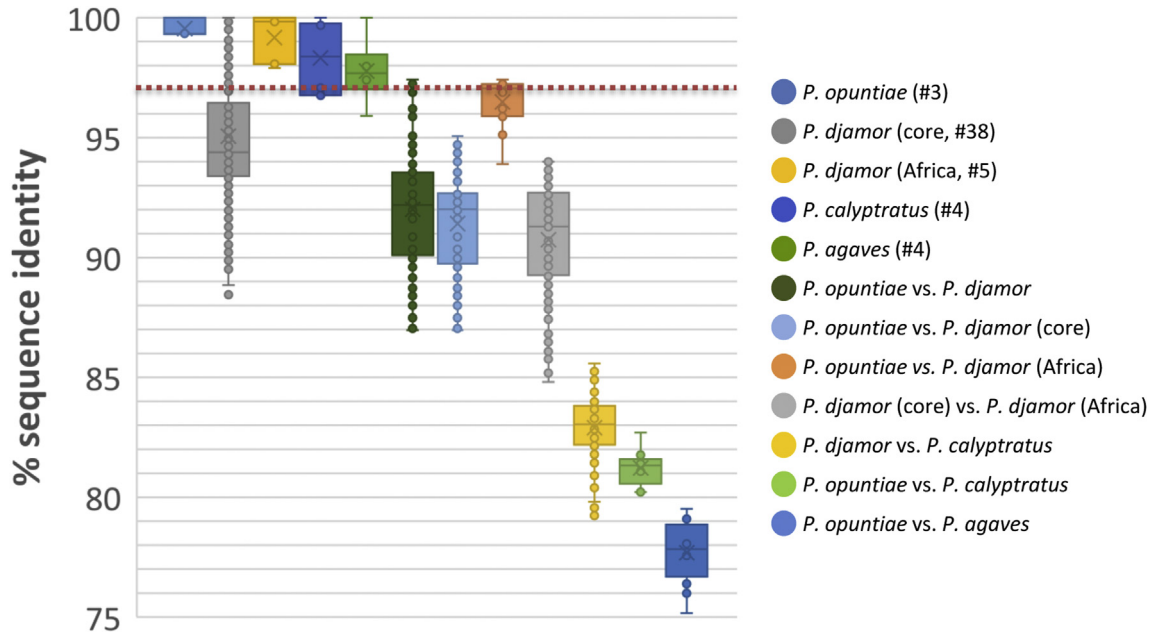

Fig. 4. Box-plot representing ITS sequence identity values within and between selected dimitic *Pleurotus* taxa. The size of each box represents 50 % of the values, the black horizontally line represents the median, the error bars the greatest and least values, while circles indicate individual values. The horizontal red dotted line represents the widely accepted 97 % threshold for delimiting species.

et al., 2010) and hereby corroborated. Therefore, the use of the name *P. opuntiae* is appropriate for describing material existing in the Mediterranean area only (i.e. in the wider region of the type locality) as it was evidenced from the outcome of this work and the analysis of pertinent Italian material.

The entire *P. djamor* complex is characterized by basidiomes of various colors (i.e. from white – off-white to tan yellowish white to pallid olive to gray to pink), with dimitic trama, and cheilocystidia extremely variable in size and shape (Camacho et al., 2012; Corner, 1981; Guzmán et al., 1993; Lechner et al., 2004; Nicholl and Petersen, 2000) and a tropical-subtropical distribution. Comparisons of spores of *P. opuntiae* (derived from the holotype and the Italian specimens hereby examined) versus those of *P. djamor* (Camacho et al., 2012; Cedano et al., 1993; Lechner et al., 2004; Segedin et al., 1995; the latter from specimens described as "P. opuntiae" but found to belong to *P. djamor* as our study shows) demonstrated that the former have larger dimensions, i.e. indicative values: 10.1 × 4.6 μm vs. 8.7 × 3.8 μm respectively. However, Nicholl and Petersen (2000) reported a much wider variability in spores of *P. djamor,* while previous studies on *P. agaves* reported spores' dimensions similar to those quoted for *P. djamor* (Camacho et al., 2012; Dennis, 1970). In general, morphological characters seem to be of limited usefulness at discriminating among dimitic taxa of the *P. djamor* complex (incl. *P. opuntiae*) and related entities (e.g. *P. agaves*).

Closely related to the *P. djamor* complex is the sister group of *P. calyptratus* (100 % and 0.98 for ITS, 100 % and 1.00 for LSU; Figs. 3 and 5a, respectively), which includes specimens occurring in temperate Eurasia (Zervakis and Balis, 1996). Although Albertó et al. (2002) introduced the name *Pleurotus djamor* f. *calyptratus* (Lindblad ex Fr.) R.H. Petersen mainly because of its dimiticity and its partial intercompatibility with *P. djamor* strains (the two taxa were included in the same intersterility group, ISG: V; Vilgalys et al., 1996), we believe that its distinct morphological/physiological features (e.g. presence of pellicular veil, absence of stipe, very slow mycelium growth rate; Zervakis and Balis, 1996), geographic distribution and molecular evidence deriving from the examination of ITS and LSU markers (noteworthy is the low ITS sequence identity value between *P. calyptratus* and *P. djamor* complex: 82.89 %; Fig. 4) are in favor of maintaining its position at the species-level.

In this study, *P. agaves* formed a well-supported clade including four ITS sequences (100 % and 1.00) all deriving from Mexico. Two of them were initially identified as "P. opuntiae" (ET3313 and TENN52368) which exemplifies the bewildering taxonomic situation previously presented. *P. agaves* was first described by Dennis (1970) from material collected in Venezuela on *Agave* sp. Later, Vilgalys et al. (1996) introduced the term *P.* "agaves" in order to refer to an incompatibility group (ISG: XI) of the genus which holds a distinct phylogenetic position in the djamor/cornucopiae clade. This was further confirmed by Huerta et al. (2010) who stated that "the taxonomic status of *P.* "agaves" needs a thorough nomenclature analysis involving morphological, genetic, and molecular studies". We agree with this statement since morphological descriptions (Camacho et al., 2012; Petersen and Krisai-Greilhuber, 1999) for material which was either reported or proved (according to the outcome of our study) to be *P. agaves* differ in certain characters, e.g. spores and cheilocystidia size and/or shape. Moreover, it is now clear that *P.* "agaves" sensu Vilgalys et al. (1996) does not include *P. opuntiae* as stated by Huerta et al. (2010) and it is not associated/related with *P. djamor* as previously reported (Camacho et al., 2012; Corner, 1981). Additional specimens under the name *P. agaves* collected from Mexico (Camacho et al., 2012; García and Guevara, 2005) and Brazil (Bononi et al., 2008; this is apparently erroneous since the basidiomes collected are monomitic) have been also studied but as it was already mentioned, their identity should be confirmed through sequence analysis to establish the distribution of this species in the Americas.

P. giganteus (Berk.) Karunarathna & K.D. Hyde is a species with prominent morphological features, e.g. broadly ellipsoid spores, wide and well separated lamellae, distinct lamellar edge with a broad sterile layer of lecythiform cheilocystidia and a metavelangiocarpic development process (Karunarathna et al., 2011), reported so far in south Asia and Australia. Although the findings of our work agree with the view expressed by Karunarathna et al.

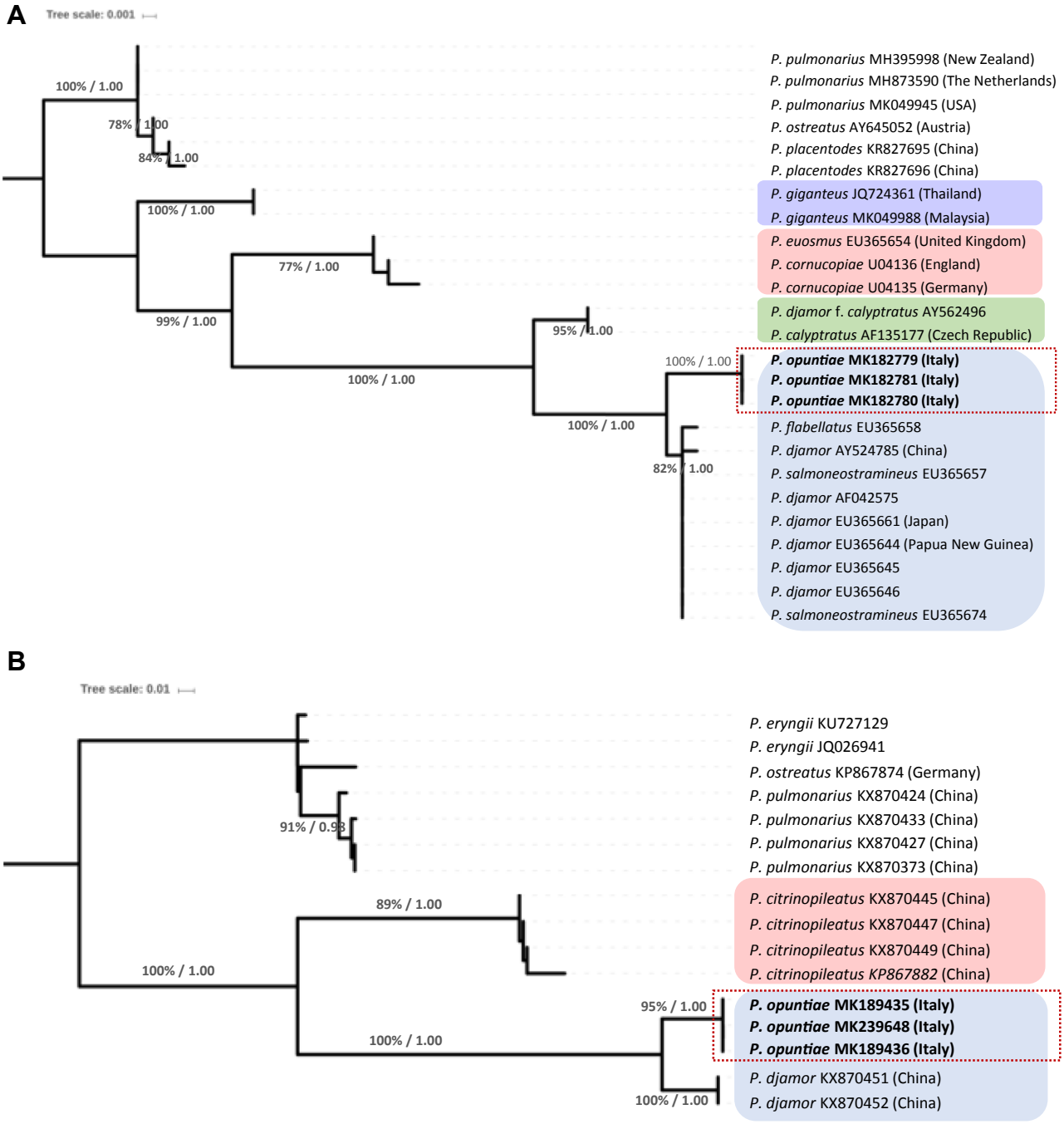

Fig. 5. Phylogenetic tree of *Pleurotus* species inferred from maximum likelihood (ML) analysis based on (A) LSU and (B) RPB2 sequence data. ML bootstrap values (BS) ≥ 70 % and Bayesian posterior probabilities (BPP) ≥ 0.95 are shown above and below the branches (or before and after the slash), respectively. Sequences generated in this study are indicated in boldface, while sequences presented on coloured background correspond to dimitic taxa.

(2011) that this fungus is better placed in *Pleurotus* than in *Lentinus* (*L. giganteus* Berk.), the phylogenetic position of *P. giganteus* lies within the core of the dimitic *Pleurotus* subclade (94 % and 1.00 support values to the group of species previously mentioned; Fig. 3) and is not closely related to taxa of the subgenus *Coremiopleurotus* as previously reported.

A cluster of taxa composed of two sister groups (91 % and 1.00; Fig. 3) was formed by *P. rickii* Bres. and the *P. cornucopiae* complex in the ITS tree. The former includes two sequences originating from Brazilian material (Menolli Jr. et al., 2014), i.e. the topotype of this species (Bresadola, 1920); *P. rickii* was also reported to occur in Argentina, and produces basidiomes with dimitic trama, well developed stipe and a conspicuous veil (Lechner et al., 2004). The latter group comprises seven sequences under three different names within a subclade of high support (97 % and 1.00; Fig. 3): *P. citrinopileatus* Singer, *P. cornucopiae* (Paulet) Rolland and *P. euosmus* (Berk.) Sacc. *P. citrinopileatus* was considered by some authors as an Asiatic morphovariant of *P. cornucopiae* forming white to bright yellow pilei, and intercompatibility results further strengthened this view (Bao et al., 2004; Ohira, 1990; Petersen and Hughes, 1993). In contrast, others reported it as a distinct species on the basis of morphological data only (Hilber, 1982; Singer, 1986). Our ITS

phylogeny produced a subclade consisting of two *P. citrinopileatus* sequences and one sequence (AF079582) of unclear origin, which is well separated from the other sister subclade including South Korean and Austrian material (the latter coincides with the geographic origin of *P. cornucopiae* epitype; Petersen and Krisei-Greilhuber, 1999). Quite interestingly, two sequences from UK specimens under the name *P. euosmus* appeared to be closely related with *P. cornucopiae* in both ITS and LSU trees (Figs. 3 and 5a). *P. euosmus* has been so far reported from England and Scotland only, and shares the same habitat preference (i.e. *Ulmus* spp.) as *P. cornucopiae* (Hilber, 1982).

Another terminal subclade was composed of five ITS sequences (100 % and 1.00; Fig. 3), three deriving from North America (USA and Mexico) and two of unknown origin, and corresponds to *P. levis* (Berk. & M.A. Curtis) Singer. Although two of these sequences were deposited as "P. dryinus" (MH211881 and AY265823), these identifications are apparently erroneous since *P. dryinus* forms a distinct phylogenetic group within the genus and includes material from Europe only (Fig. 3). However, *P. levis* is morphologically similar to *P. dryinus* by exhibiting centrally stipitate pilei with decurrent lamellae and a partial veil. On the other hand, they are distinguished mainly by the formation of tan to brown chlamydospores in cultures of *P. dryinus*; cultures consist of generative hyphae only and present relatively slow growth rates (Petersen et al., 1997). It has also to be noted that this species should be transferred from *Lentinus* [*Lentinus levis* (Berk. & M.A. Curtis) Murrill; currently under this name in Mycobank and Index Fungorum] to *Pleurotus* since characters typical of the latter genus are present, e.g. formation of nematotoxic microdroplets in cultures and on basidiomes, and yellow coloration near the pileus margin upon drying of the typically white to pallid pink basidiomes. Moreover, the phylogenetic analysis conducted in our study evidenced its affinity to the genus *Pleurotus*.

In conclusion, the phylogenetic analyses presented in this study provide an insight to the relationships among *Pleurotus* taxa with emphasis on the *P. djamor* complex, and contributes to the elucidation of several ambiguous taxonomic entities. Especially as regards dimitic species, *P. opuntiae* is confined in the Mediterranean area only; specimens under this name from other regions (i.e. Mexico, Asia, New Zealand) belong to *P. djamor*. The latter also includes material labeled with various other epithets (e.g. *P. flabellatus*, *P. ostreatoroseus* and *P. salmoneostramineus*). In addition, *P. calyptratus*, *P. agaves*, *P. rickii* and *P. levis* are valid species, *P. euosmus* appears to be conspecific with *P. cornucopiae*, while some specimens identified as *P. eous* form a distinct entity within a well-supported cluster of monomitic *Pleurotus* taxa. Further work on additional, properly documented material is needed to assess the distribution range and/or confirm the status of taxa within the *P. cornucopiae* complex, of *P. agaves*, *P. eous*, *P. parsonsiae*, and of African isolates identified either as *P. djamor* or as *P. opuntiae*.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2018.12.005.

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