# WHY *OMPHALOTUS ILLUDENS* (SCHWEIN.) BRESINSKY ET BESL IS AN INDEPENDENT SPECIES

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**ABSTRACT. Why *Omphalotus illudens* (Schwein.) Bresinsky et Besl is an independent species.** The genus *Omphalotus* is known from tropical or temperate areas all over the world. Currently, eight species are described. Two of them, *O. olearius* and *O. illudens*, occur in Europe. Since these two species are morphologically very similar, their taxonomical status is still controversial. This study provides a careful review of the problem: morphological, chemotaxonomical, molecular data as well as data from mating experiments are evaluated. The results point to the conclusion that *O. olearius* and *O. illudens* are distinct species.

**Key words:** *Omphalotaceae*, *Omphalotus olearius*, *Omphalotus illudens*, taxonomy.

**RESUMEN. Por qué *Omphalotus illudens* (Schwein.) Bresinsky et Besl es una especie independiente.** El género *Omphalotus* es conocido de la zona tropical a la templada, en todo el mundo. Actualmente, hay ocho especies descritas. Dos de ellas, *O. olearius* y *O. illudens* viven en Europa. Ya que estas dos especies son morfológicamente muy similares, aún existe discusión sobre su estatus taxonómico. Este estudio proporciona una revisión detallada de este problema: los datos morfológicos, quimiotaxonómicos, moleculares, al igual que experimentos de cruzamiento, son sometidos a evaluación. Los resultados llevan a la conclusión de que *O. olearius* y *O. illudens* son dos especies distintas.

**RESUM. Per qué *Omphalotus illudens* (Schwein.) Bresinsky et Besl és una espècie independent.** El gènere *Omphalotus* viu des de la zona tropical a la temperada de tot el món. Actualment, hi ha vuit espècies descrites. Dues, *O. olearius* i *O. illudens* viuen a Europa. Tenint en compte que aquestes dues espècies són morfològicament molt semblants, el seu estatus taxonòmic és encara matèria de controvèrsia. Aquest estudi es basa en una revisió detallada d'aquest problema: s'hi evaluen dades morfològiques, quimiotaxonòmiques, moleculars, i també experiments d'encreuament. Els resultats ens porten a la conclusió que *O. olearius* i *O. illudens* són dues espècies diferents.

## INTRODUCTION

There have long been controversial opinions among European mycologists about the interpretation of *Omphalotus illudens* (Schwein.) Bresinsky et Besl which was originally described by SCHWEINITZ (1822) from North Carolina, USA. Some authors treat *O. illudens* and *Omphalotus olearius* (DC.: Fr.) Singer as conspecific (PEGLER, 1977; WATLING & GREGORY, 1989), others separate the two taxa (KUYPER, 1995; KIRCHMAIR *et al.*, 2002). Recently, ORTEGA *et al.* (2000) recombined *O. illudens* as a variety of *O. olearius*. Most of these interpretations are based exclusively on morphological observations and the potential geographic distribution of these two taxa in Europe: *O. olearius* is considered as a Mediterranean species while *O. illudens* has a more northern distribution (e.g. Belgium, northern France, Netherlands). Because of natural variability of morphological characters as well as their different weighting by mycologists, the distinction of species is often subject to relatively large uncertainties. This is especially true for species that are morphologically as similar as *O. illudens* and *O. olearius*. Meanwhile, it is well established that – at least in such cases – the application of additional methods (e.g. chemotaxonomy, DNA-analyses) may lead to a more reliable solution of such problems. The objective of this study was the evaluation of published data sets obtained by different taxonomic methods whereby new data on morphology and from DNA-sequencing of the ITS1-5.8S-ITS2 rDNA region were added.

## METHODS

Microscopic descriptions were made from sections or pieces of tissue taken from dried basidiomata, which were mounted in KOH 3% for spore measurements or in *aqua dest.* for pigment observations in the pileipellis. Spore measurements were made from video print images (CCP Color Video Camera Module, Sony Multiscan UP-930). Spore size is given in the form (min.) mean ± standard deviation (max.); V = approximated volume [sample size (n) for each collection = 30]. Statistical analysis were performed by SPSS 10.1. Mean values and variances were analysed with the unpaired student's t-test ($P < 0.05$) and with the Levene-test, respectively. Comments on basidiomatal colouration were based on fresh and/or dried specimens, photographs, and/or descriptions.

DNA was extracted from dried specimens or cultures by mechanical lysis using CTAB (cetyltrimethylammonium bromide) following standard procedures (ZOLAN & PUKKILA, 1986). Primers for PCR amplification and for sequencing of the ITS1-5,8S-ITS2 region were ITS1 and ITS4 (WHITE *et al.*, 1990). For detailed description of methods see MORANDELL (2001) and STOLZ (1998). The sequences obtained are deposited in the National center for Biotechnology Information (NCBI) GenBank under the accession numbers AF525042-AF525061. Alignment was initially carried out by using the computer program ClustalW 1.81 and manually adjusted in the data editor of PAUP* 4.0b8 (SWOFFORD, 1998). The phylogenetic analysis was performed with PAUP* 4.0b8. *Nothopanus eugrammus* (Mont.) Singer was chosen as outgroup (GenBank: AF525074, AF525075). Maximum parsimony was used to search for optimal trees with the following settings: MULPARS = on, steepest descent not in effect, MAXTREES = 10000, branch-swapping algorithm = tree-bisection-reconnection (TBR), and gaps treated as "fifth base". The trees were found by random sequence addition: 100 heuristic searches were performed, the shortest trees over all replicates were kept and assumed to be the most parsimonious reconstructions. Support for branches was calculated with the Bootstrap method (heuristic search, bootstrap replicates = 100; gaps treated as "fifth base", addition sequence random, one tree held at each step during stepwise addition, TBR on, MAXTREES = 10000).

## MATERIAL EXAMINED

*Omphalotus illudens* (Schwein.) Bresinsky et Besl:

**BELGIUM:** Liège, Nivezé, 20-9-1997, *leg.* J. Prados, BR 70376,51.- Namur, Briquemont-Rochefort, 13-9-1981, *leg.* P. Heinemann, BR 21676,45.- Namur, Ciergnon-Briquemont, 8-9-1987, *leg.* P. Heinemann, BR 2704,85.- **FRANCE:** Chamboulive, Corrèze, 1-10-1980, *leg.* L. Thumas, BR 18303,67.- **USA:** Bethesda MD, 7422 Hampton Lane, around stumps of hardwood trees, 7-9-1967, *leg.* Ann Tenks, OKM 6086.- Michigan, Ann Arbor, 20-10-1973, *leg.* A.H. Smith, OKM 9597.- California, San Mateo Co., Spring Valley Lakes, *leg.* R.T. Orr and D.B. Orr, VT 452 (culture).– North Carolina, Durham, 14-8-2000, *leg.* A. Ganley, *det.* M. Kirchmair, IB 20000961.

*Omphalotus olearius* (D.C.: Fr.) Singer:

**CROATIA:** Mali Lošinj, Čikat Bay, on *Pinus halepensis*, 5-10-1997, *leg.* and *det.* M. Kirchmair, IB 19970604.- Mali Lošinj, Čikat Bay, under *P. halepensis*, 5-10-1997, *leg.* and *det.* M. Kirchmair, IB 19970605.- Veli Lošinj, St. Juan, on soil (roots?) between *P. halepensis*, 6-10-1997, *leg.* and *det.* M. Kirchmair, IB 19970776.- Mali Lošinj, Čikat Bay, on *P. halepensis*, 9-10-1997, *leg.* and *det.* M. Kirchmair, IB 19970777.- Mali Lošinj, Čikat Bay, on *Quercus ilex*, 9-10-1997, *leg.* and *det.* M. Kirchmair, IB 19970778.- Aerport Lošinj, Čunski Bay, on *Cistus* sp., 7-10-1997, *leg.* and *det.* M. Kirchmair, IB 19970779.- **FRANCE:** Port Man, Ile de Cros, Var, on *Q. ilex,* 31-10-1978, *leg.* and *det.* M. Moser, IB 19780492.- **GREAT BRITAIN:** England; Oxford, 12-1973, *leg.* and *det.* E. Jones, CBS 332.85 (culture).- **HUNGARY:** Budapest, 7-1955, *leg.* and *det.* G. Bohus, CBS 163.55 (culture).- **ITALY:** Prov. Parma, Monte Penna, on stump of *Castanea sativa*, 12-10-1994, *leg.* and *det.* M. Kirchmair and R. Pöder, IB 19940903.- Prov. Parma, Monte Penna, on stump of *C. sativa*, 12-10-1994, *leg.* and *det.* M. Kirchmair and R. Pöder, IB 19940904.- Prov. Parma, Marzocco, on *Quercus* sp., 6-10-1996, *leg.* and *det.* M. Kirchmair, IB 19960674.- Sicilia, Fascio tre, under *Quercus cerris*, 13-11-1998, *leg.* R. Piérart, BR 96550,35.- Avezzano, 24-10-1997, *leg.* and *det.* M. Moser, IB 19970780.- Sardinia, Tempio vs. Palau, under *Quercus suber* and *Cistus* sp., 31-10-2000, *leg.* and *det.* H. Ladurner, IB 20000311.- **SPAIN:** Granada, La Alcaicería, urbanización Cortijos de Valparaíso, under *Q. ilex* subsp. *ballota* and *Cistus laurifolius*, 5-11-1990, *leg.* A. Ortega, M.T. Vizoso and M. Zea, *det.* A. Ortega, GDAC 36389.- Cádiz, strada da Puerto de Galiz ad Alcalá de los Gazules, Parque Natural de los Alcornocales, on old, rotten stumps in a *Q. ilex* subsp. *ballota and Q. suber* forest, 16-11-1987, *leg.* A. Ortega, M.T. Vizoso and M. Zea, *det.* A. Ortega, GDAC 31408.- Casa forestal de Bolones, Parque Natural

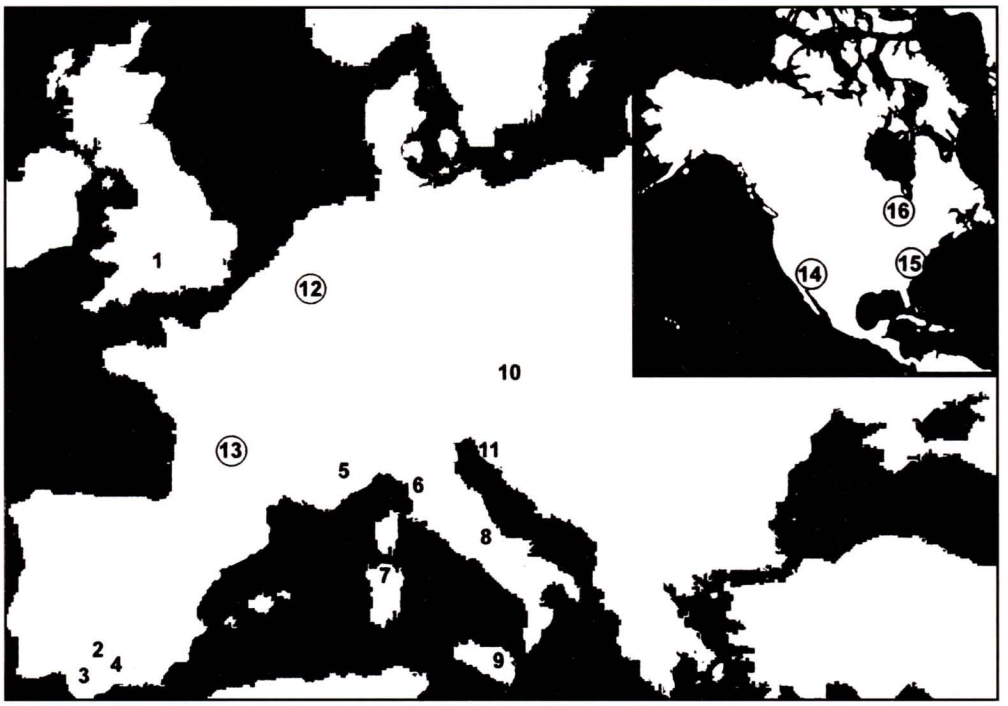



**Fig. 1.** Geographical origin of examined collections of *Omphalotus olearius* and *O. illudens* (reference numbers encircled). 1 = CBS 332.85. 2 = GDAC 30870. 3 = GDAC 31408. 4 = GDAC 36389, GDAC 42712, GDAC 42697, GDAC 43143. 5 = IB 19780492. 6 = IB 19940903, IB 19940904, IB 19960674. 7 = IB 20000311. 8 = IB 19970780. 9 = BR 96550,35. 10 = CBS 163.55. 11 = IB 19970776, IB19970777, IB 19970778, IB 19970779, IB 19970604. 12 = BR 21676,45, BR 70376,51, BR 2704,85. 13 = BR, 18303,67. 14 = VT 452. 15 = IB 20000961, OKM 6086. 16 = OKM 9597.

de la Sierra de Huétor, under *Q. ilex* subsp. *ballota*, 12-10-1996, *leg.* and *det.* A. Ortega, GDAC 42712.- Ciudad de Huétor Santillán, on soil under *Ulmus* sp., *Draca?ena* sp., *Magnolia* sp., 19-10-1998, *leg.* and *det.* A. Ortega, GDAC 42697.

*Omphalotus olearius* var. *illudens* (Schwein.) A. Ortega et Esteve-Rav.:

**SPAIN:** Córdoba, Cabra, Mojón de Cabra, on soil under *Quercus ilex* subsp. *ballota*, 23-11-1989, *leg.* A. Ortega, M.T. Vizoso and M. Zea, *det.* A. Ortega, GDAC 30870.- Granada, La Alcaicería, urbanización Cortijos de Valparaíso, on soil under *Q. ilex* subsp. *ballota*, 15-11-1998, *leg.* A. Capilla, GDAC 43143.

*Nothopanus eugrammus* (Mont.) Singer:

**USA:** Puerto Rico, Angelito Trail to Rio Mameyes, on wood, 5-6-1997, *leg.* and *det.* R. Vilgalys, DUKE 3980.- **AUSTRALIA:** Northern Territory, Darwin Botanical Garden, on wood in planted vine forest, 13-2-1995, *leg.* and *det.* R. Vilgalys, DUKE 2581.

## RESULTS

MORPHOLOGICAL OBSERVATIONS. Morphological characters of 6 collections of *O. illudens* from Europe and the USA as well as 16 collections of *O. olearius* from Croatia, France, Italy, and Spain were examined. The geographical origin of collections is pictured in Fig. 1. Macroscopically, there were only few differences between the two taxa: whereas, due to natural variability, basidiomatal shapes and sizes (diameter of pileus in relation to stipe length and width) did not provide reliable separation features, it could be observed that all *O. illudens* basidiomata were at least slightly papillate or, more often, distinctly umbonate at the center of the pileus. This feature could never be observed in *O. olearius*. Like shape and size, basidiomatal coloration varied remarkably between collections: pileus colors ranged from deep yellow to dark reddish brown in *O. olearius* and were deep yellow to yellowish orange in *O. illudens* (Figs. 2-5).

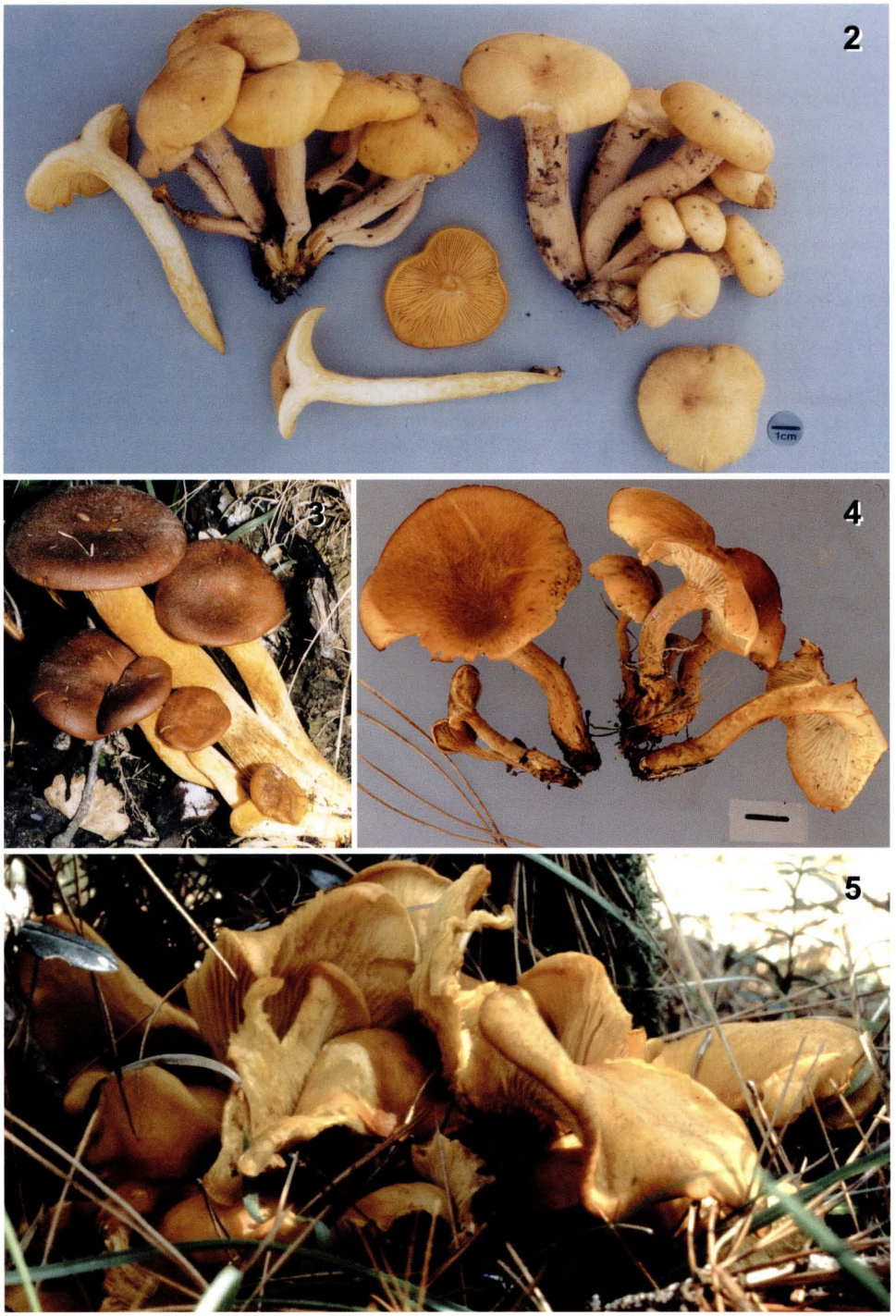
Microscopical examination revealed the following differences: (i) numerous refractive hyphae could be found in the pileipellis of *O. illudens* but were rare or absent in *O. olearius* and (ii) the basidiospores of *O. illudens* were significantly smaller (Fig. 6). The spore size of *O. illudens* (pooled values from 6 collections; n = 180) was (4.2) 5.1 ± 0.4 (5.9) × (3.8) 4.6 ± 0.3 (5.5) µm; volume (35) 58 ± 12 (92) µm$^3$ and from *O. olearius* (4.8) 6.4 ± 0.6 (8.4) × (4.6) 5.8 ± 0.5 (8.0) µm; volume (54) 112 ± 29 (280) µm$^3$ (16 collections; n = 480). All of these mean values were significantly different (p = 0.001) for the two taxa.

SEQUENCE ANALYSES OF THE ITS1-5.8S-ITS2 RDNA REGION. Six collections of *O. illudens* and 14 collections of *O. olearius* were analysed. The total alignment covered a length of 660 characters. After excluding gaps and areas of ambiguity 632 characters remained for analysis. 460 characters were constant, 51 variable characters were parsimony-uninformative, and 121 were parsimony-informative. Identical sequences were pruned from analysis, thus twelve different DNA-sequences of the ITS1-5.8S-ITS2 rDNA region were analysed for the two ingroup taxa. A heuristic search of the 14 sequences (outgroup: *Nothopanus eugrammus*) yielded two equally parsimonious trees of 211 steps (consistency index (CI) = 0.9621, retention index (RI) = 0.9652, rescaled consitency index (RC) = 0.9286, and a homoplasy index = 0.0379). The tree is divided into two major clades: all *O. illudens* isolates form one group with a bootstrap support of 100%. The *O. olearius* branch is supported by 100% likewise (Fig. 7).

## DISCUSSION

EVALUATION OF MORPHOLOGY. Among the classical characters used by both the "splitters" and "lumpers", basidiomatal shape, size, and coloration seem to have played a crucial role. However, due to differing growth conditions (e.g. temperature, humidity, host diversity) these characters are obviously variable and overlapping. Therefore, the use of such features for the limitation of discrete taxonomic units often leads to vicious circles. So, for example, it is not surprising that no different spore sizes can be found when pale and slender forms of *O. olearius* are compared with its short-stiped, darker colour variants. Therefrom, one cannot conclude that *O. illudens* - slender and relatively pale basidiomata are features generally assigned to this taxon - is conspecific with *O. olearius*. However, it is well accepted that "good" morphospecies must differ from each other in at least two independent characters (KUYPER, 1986). This is the case for the two taxa in question: the combination of an umbonate or papillate pileus, numerous refractive hyphae in the pileipellis (KUYPER, 1995), and distinctly smaller spores (KIRCHMAIR *et al.*, 2002) allow the delimitation of *O. illudens* from *O. olearius* at the morphological level. The spore sizes of the two Spanish collections on which ORTEGA *et al.* (2000) based their combination *O. olearius* var. *illudens* (Schwein.) A. Ortega et Esteve-Rav. perfectly fit with typical *O. olearius* values from 14 other south European collections; refracting hyphae in the pileipellis were rare and a papilla or umbo could not be observed.

EVALUATION OF CHEMOTAXONOMICAL DATA. *Omphalotus* species produce a number of typical Boletales pigments (BRESINSKY & BESL, 1979; KIRCHMAIR *et al.*, 2002) like variegatic acid, xerocomic acid, and atromentin. Comparative thin layer chromatography (TLC) revealed little differences in pigment patterns of *O. olearius* and *O. illudens* when dried basidiomata were extracted.




**Figs. 2-5. 2.** *Omphalotus illudens* (North Carolina, USA; IB 2000/0961). **3-5.** *O. olearius* from Italy (**3.** IB 1994/0904) and from Croatia (**4.** IB 1997/0604; **5.** IB 1997/0778). Photos: U. Peintner, E. Steiner, P. Holzknecht.

Pigment patterns from culture extracts, however, were clearly different: compared with *O. olearius*, eight additional bands could be detected in *O. illudens*; two bands were lacking (KIRCHMAIR *et al.*, 2002). The pileipellis of both species stains greenish when treated with 30 % KOH, but this reaction, which is correlated with the atromentin content, could be found in all *Omphalotus* species.

MATING EXPERIMENTS. Mating studies by PETERSEN & HUGHES (1998) who paired, beside others, monokaryon cultures of *O. illudens* from North America and *O. olearius* from southern Europe showed that *O. illudens* "is almost sexually isolated from the European *O. olearius*".

EVALUATION OF MOLECULAR DATA. The results of restriction fragment length polymorphism (RFLP) analyses of the ribosomal ITS1-5.8S-ITS2 region by HUGHES & PETERSEN (1998) clearly separated *O. illudens* from *O. olearius*. The rDNA-sequence analyses of the ITS1-5.8S-ITS2 region presented in this study reveal a clear separation of the two taxa: all *O. illudens* collections, regardless of their geographic origin, form one clade supported by a bootstrap value of 100 %. In this context it should be mentioned that four collections, two from the USA and two from Europe, exhibit an identical sequence. Equally, all of the 14 examined *O. olearius* collections are grouped in a second clade (bootstrap support 100 %). These collections comprise light and dark colour variants from Croatia, France, Great Britain, Hungary, and Italy; no correlation between sequences and colouration of basidiomata can be observed.

## CONCLUSION

Evidences derived from different, independent taxonomical data sets - morphology, chemotaxonomy, mating experiments, and DNA data - strongly support the conclusion that *O. illudens* and *O. olearius* are distinct species.

## AKNOWLEDGEMENT

Curators and staff of the various culture collections that provided material for this study are sincerely thanked. We wish to extend our thanks to U. Peintner for methodical assistance with sequencing and providing photographs, and to C.C. Teissier de Wanner for the translation of the abstract to Spanish. This work is dedicated to Mr. August Rocabruna on occasion of his 80$^{th}$ birthday.

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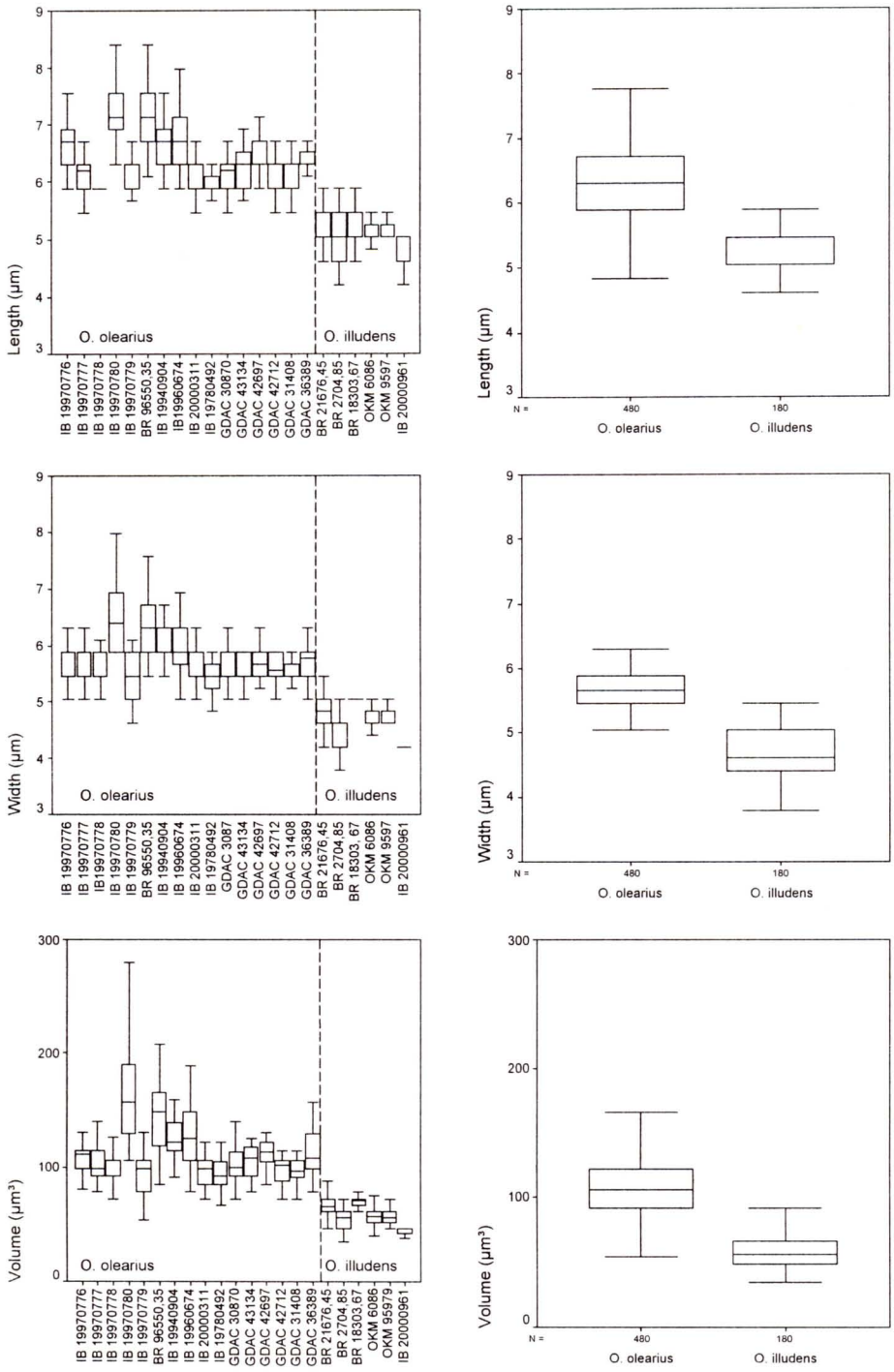


**Fig. 6.** Spore sizes of *Omphalotus olearius* and *O. illudens* in box and whisker plots (50% of values and median within boxes; whiskers show minimum and maximum values). The means of pooled values of spore length, width, and volume (graphs on the right) are significantly different ($p = 0.001$).

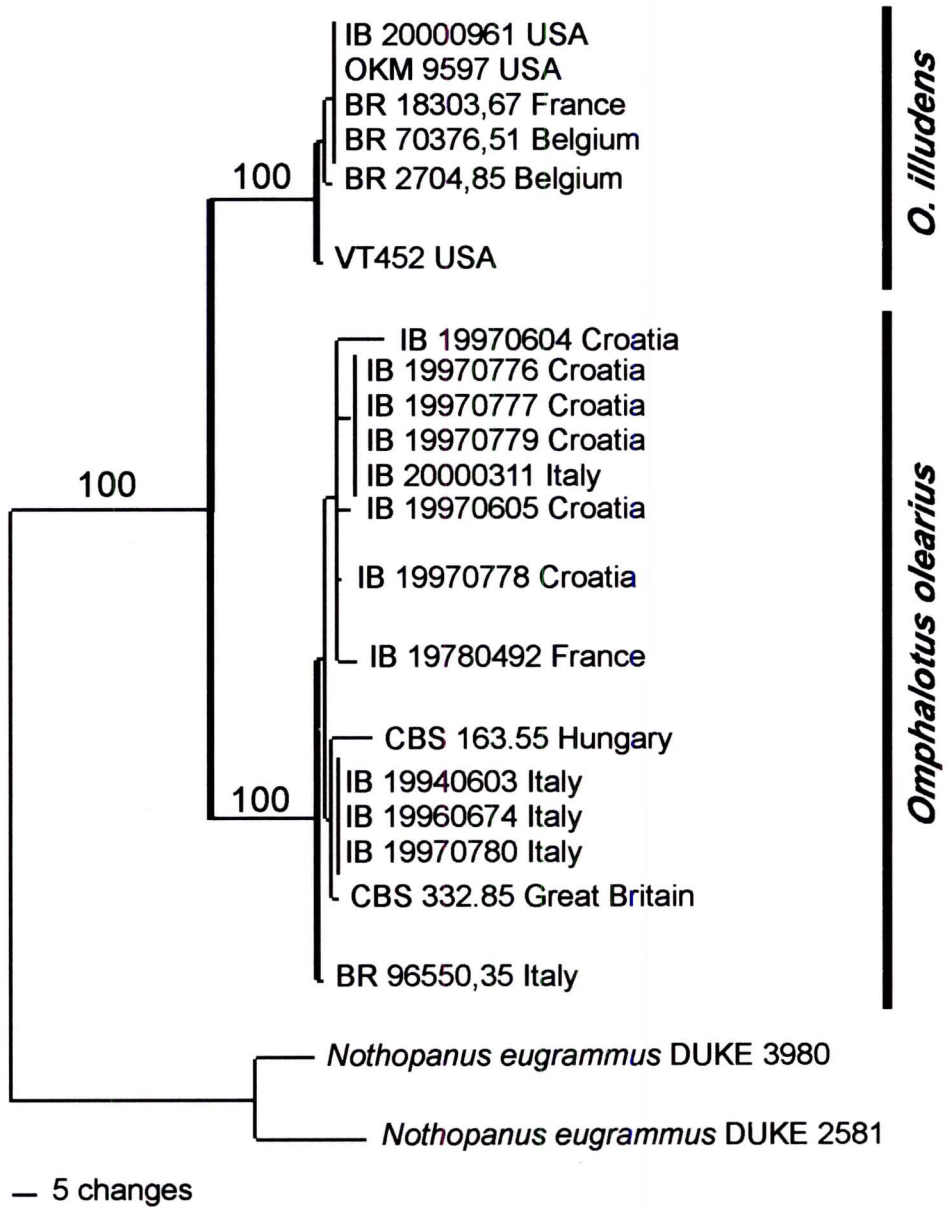


**Fig. 7.** Phylogram of one of two most parsimonious trees of 211 steps based on ITS-sequences: CI = 0.9621, RI = 0.9652. Bootstrap values are shown above branches.

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