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CHEMOTAXONOMICAL AND MORPHOLOGICAL OBSERVATIONS IN THE GENUS OMPHALOTUS (OMPHALOTACEAE)

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Comparative thin-layer chromatography – for the first time applied to *Omphalotus olivascens* var. *olivascens*, *O. olivascens* var. *indigo*, *O. nidiformis*, and to *O. mexicanus* – revealed strikingly similar pigment patterns for all *Omphalotus* species except *O. mexicanus*. Atromentin, thelephoric acid and pulvinic acid derivates were found in dried material and/or culture extracts of all species. Illudin S and illudin M were detected in *O. mexicanus* by high-performance liquid chromatography-atmospheric pressure chemical ionisation mass spectrometry. Data on morphological features of all described *Omphalotus* and *Lampteromyces* species are listed, illustrated, and summarized in a key. New combinations in the genus *Omphalotus* are proposed for *Lampteromyces japonicus* and *L. mangensis*.

In classical fungal taxonomy morphological-anatomical characters are often used exclusively to classify specimens. Unfortunately, many fungi show only few 'good' morphological characters. Therefore, if possible, additional methods should be applied to elucidate their taxonomic relationships. Besides eco-physiological (e.g. host specificity) or molecular (e.g. DNA sequences) characters the investigation of pigment patterns still helps to clarify discrete taxonomic limits. Following Singer (1986) the order Boletales is mainly characterized by the occurrence of "pigments of the variegatic acid type or derivates (or otherwise related to pigments commonly found in boletes)". The detection of certain Boletales pigments in members of the genera *Omphalotus* Fayod and *Lampteromyces* Sing. was one of the main motives of Bresinsky & Besl (1979b) to include them into the Paxillaceae. Further investigations (Kämmerer et al., 1985) focused on physiological characters of some species of the afore mentioned genera revealed relevant differences to other members of the Paxillaceae: based on both the occurrence of sesquiterpenes of the illudane type and the formation of so called 'white soft-rot', features not found in other members of the Paxillaceae, the new family Omphalotaceae Bresinsky was established. Until recently most chemotaxonomical studies in the genus *Omphalotus* were focused on two species only: *O. olearius* and *O. illudens*. Therefore, the objective of the present study was to gather additional chemotaxonomical and morphological data aiming at an improvement of the taxonomic structure of the genus which is still unclear and incomplete.

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METHODS

Microscopic descriptions were made from sections or pieces of tissue taken from dried basidiomata, mounted in 2.5% KOH (pigment topology was studied in aqua dest. because most of the pigments are soluble in KOH). A Nikon Labophot microscope with an automatic photographic system (Sony Multiscan Video Printer UP-930) was used for light microscopy (LM). Drawings of anatomical features are based on video prints. Video prints of spores taken from a spore print or from the stipe were used for measurements also (sample size for each collection > 30). Measurements are given in the form (minimum) mean ± standard deviation (maximum). Colour notations are mainly based on original descriptions.

For thin layer chromatography (TLC) the different strains were cultivated on MEA plates (malt extract agar, Merck 5398) for 20 days at 20 °C and two hours light per day. The cultures (each with 20 ml medium) were liquefied at about 90 °C and extracted with hot ethanol acidified with a few drops of 1N HCl. This raw extract was shaken out three times in a separating funnel with an equal amount of pure ethyl acetate. The ethyl acetate phase was dried with anhydrous sodium sulfate, filtrated and evaporated to dryness at 50 °C. The residue was resolved in 1.5 ml ethyl acetate. Twenty µl of these extracts were spotted on silica gel TLC-plates (Merck 1.05721) with toluene : ethyl formate : formic acid (10 : 5 : 3) as a solvent according to Bresinsky (1974). If bands were too weak for characterization, the amount of spotted extracts was increased to 30 or 40 µl. For separating thelephoric acid, methyl ethyl ketone : H_2O : formic acid (250 : 25 : 1) was used as solvent. Pigment patterns were examined in daylight and under UV (366 nm). For determining pulvinic acid derivates a $K_3[Fe(CN)_6]/NaHCO_3$ solution was used as spray reagent: variegatic acid and xerocomic acid change their colour from yellow to blue. Incubating the chromatogram in ammonium vapour for half an hour changes the colour of these two pigments to red. Atromentinic acid shows no colour change with $K_3[Fe(CN)_6]/NaHCO_3$ but turns violet with ammonium (Bresinsky, 1974). Thelephoric acid was identified following Bresinsky & Rennschmied (1971). For reference the following compounds were used: xerocomic acid and variegatic acid isolated from *Boletinus cavipes*, thelephoric acid from *Thelephora terrestris*, atromentin from *Paxillus atrotomentosus*, and atromentinic acid provided by Dr. H. Besl (Regensburg, Germany). Isolation procedures were carried out following Kögl & Postowsky (1924), Bresinsky & Orendi (1970), and Bresinsky & Rennschmied (1971).

For detection and identification of illudin S and illudin M by high-performance liquid chromatography – atmospheric pressure chemical ionisation – mass spectrometry (HPLC-APCI-MS) liquid cultures of *O. mexicanus* were used according to Kirchmair et al. (1999). Illudin S and illudin M standards were kindly provided by Trevor C. McMorris, San Diego, USA. The HPLC system consisted of a low-pressure gradient micro pump (model Rheos 2000, Flux Instruments, Karlskoga, Sweden) controlled by a personal computer, a vacuum degasser (Knauer, Berlin, Germany), and an injector (model Cheminert C3-2006, Valco Instruments Co. INC., Houston, TX, USA) with a 5 µl sample loop. LC separations were performed with a gradient of 10–42% acetonitrile in 0.1% aqueous acetic acid in 20 min at a flow-rate of 250 µl/min. The Nucleosil ODS (3 µm, 100 Å) column packing material was obtained from Macherey & Nagel (Düren, Germany) and packed into a 150 × 2 mm i.d. stainless steel column (Grom, Herrenberg, Germany) with the help of an air-driven high-pressure packing pump (Knauer, Berlin, Germany).

APCI-MS3 was performed on a Finnigan LCQ quadrupole ion trap mass spectrometer (Finnigan MAT, San Jose, CA, USA) equipped with the atmospheric pressure chemical ionisation ion source. For LC-MS analysis with heat assisted pneumatic nebulisation, a corona current of 5 µA at 5 kV and a sheath gas flow of 60 arbitrary units were employed. The temperatures of the heated nebuliser and the heated pileusillary were set at 450 and 200 °C, respectively. Highly selective MS^3 mass spectra for illudin S and illudin M were obtained using the transitions 247.1→217.1→full scan from 60–250 u and 231.1→213.2→full scan from 60–250 u, respectively, with a relative collision energy of 15%. Total ion chromatograms and mass spectra were recorded on a personal computer with the Navigator software version 1.2 (Finnigan).

Material studied

Cultures. For a list of examined cultures see Table I.

Omphalotus illudens (Schwein.) Bresinsky & Besl.

Collections examined. BELGIUM: Liège, Nivezé, 20 Sept. 1997, *J. Prados*, BR 70376,51; Namur, Briquemont-Rochefort, 13 Sept. 1981, *P. Heinemann*, BR 21676,45; Namur, Couvin, 22 Sept. 1977, *C. Cnobs*, BR 12717,10, BR 12714 ,09; Namur, Ciergnon-Briquemont, 8 Sept. 1987, *P. Heinemann*, BR 2704,85. — USA: Bethesda MD, 7422 Hampton Lane, around stumps of hardwood trees, 7 Sept. 1967, *Ann Tenks*, OKM 6086; Michigan, Ann Arbor, 20 Oct. 1973, *A.H. Smith*, OKM 9597.

Omphalotus mexicanus Guzmán & Mora.

Collections examined. MEXICO: Sierra del Tigre, Región de Mazamitla, carretera de Tamazula-Jiquilpan, Jalisco, 23 June 1983, *G. Guzmán*, XAL 23474; Cerca de San Cristobal de Las Casas, Chiapas, 23 June 1983, *G. Guzmán*, XAL 29252.

Omphalotus nidiformis (Berk.) O.K. Mill.

Collection examined. AUSTRALIA: NSW, Blue Mts, Mt Wilson, on bark of living trees in the rain forest, 7 Apr. 1983, *E. Horak*, ZT 2144.

Omphalotus olearius (D.C.: Fr.) Sing.

Collections examined. AUSTRIA: near Vienna, on hardwood trees, 1 Oct. 1952, *M. Moser*, IB 1952/115. — CROATIA: Veli Losinj, St. Juan, on soil (roots?) between *Pinus halepensis*, 6 Oct. 1997, *M. Kirchmair*, IB 1997/776; Mali Losinj, Èikat Bay, on *Pinus halepensis*, 9 Oct. 1997, *M. Kirchmair*, IB 1997/777; Mali Losinj, Èikat Bay, on *Quercus ilex*, 9 Oct. 1997, *M. Kirchmair*, IB 1997/778; Aerport Losinj, Èunski Bay, on *Cistus sp.*, 7. Oct. 1997, *M. Kirchmair*, IB 1997/779. — FRANCE: Port Man, Ile de Cros, Var, on *Quercus ilex*, 30 Oct. 1977, *M. Moser*, IB 1977/222; Port Man, Ile de Cros, Var, on *Quercus ilex*, 31 Oct. 1978, *M. Moser*, IB 1978/492. — ITALY: Prov. Parma, Monte Penna, on stump of *Castanea sativa*, 12 Oct. 1994, *M. Kirchmair, R. Pöder*, IB 1994/904; Prov. Parma, Marzocco, on *Quercus sp.*, 6 Oct. 1996, *M. Kirchmair*, IB 1996/674; Sicilia, Fascio tre, under *Quercus cerris*, 13 Nov. 1998, *R. Piérart*, BR 96550,35; Aressano, 24 Sept. 1997, *M. Moser*, IB 1997/780. — KENYA: Mt Elgon, under olive trees, 8 May 1964, *P.H. Erwin*, K 50515. — TANZANIA: Magamba Forest Reserve, Lushoto, W. Usambora Mts, Tanga Prov., on decaying wood, 23 Apr. 1968, *D.N. Pegler*, K 50514.

Omphalotus olivascens var. *indigo* Moreno, Esteve-Rav., Pöder & Ayala.

Collections examined. MEXICO: Baja California Norte, Santa Rosa, on stump of *Quercus agrifolia*, 10 Nov. 1993, *G. Moreno*, AH 16295; Baja California Norte, San Antonio de las Minas, on stump of *Quercus agrifolia*, 14 March 1990, *G. Moreno*, AH 13187; Baja California Norte, Las Lomas, San Antonio de las Minas, on stump of *Quercus agrifolia*, 2 Feb. 1996, *G. Moreno*, IB 1996/731.

Omphalotus olivascens var. *olivascens* Bigelow, O.K. Mill. & Thiers.

Collections examined. USA: California, San Mateo Co., Junipero Serra Park, San Bruno, on oak stumps, 4 Dec. 1960, *H.D. Thiers*, HDT 8547; California, Cleveland National Forest, Decker Canyon, on a stump of *Quercus agrifolia*, 12 March 1984, *O.K. Miller Jr.*, OKM 20911.

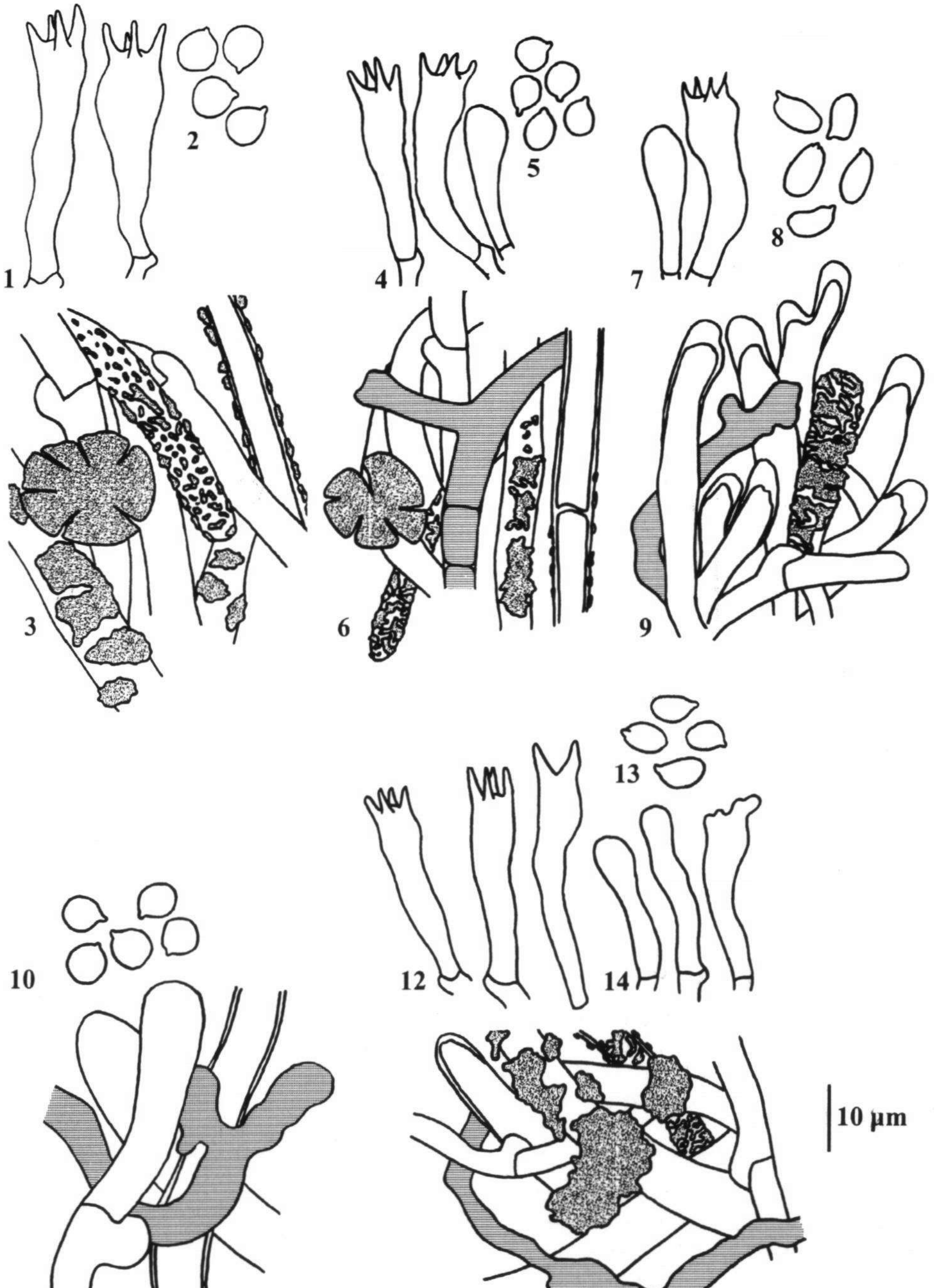
Omphalotus subilludens (Murrill) Bigelow.

Collection examined. USA: Austin, Texas, on hardwood, 3 Nov. 1983, *O.K. Miller Jr.*, OKM 20850.

RESULTS

Morphological observations

Relevant morphological characters and observations are discussed here and presented in Tables II & III and in Figs. 1–34.



Figs. 1–15. Microanatomical features of *Omphalotus* taxa: basidia and basidioles, basidiospores, cheilocystidia (14), and elements of the pileipellis. *Omphalotus olearius* (1–3), *O. illudens* (4–6), *O.* aff. *olearius*, 'Kenya collection' (7–9), *O.* aff. *olearius*, 'Tanzania collection' (10–11), and *O. subilludens* (12–15). Refractive hyphae (light grey) and pigment incrustations (dark grey) are highlighted by shadings.

Table I. List of cultured strains studied. Acronymes: CBS = Centraalbureau voor Schimmelcultures, Baarn, The Netherlands; VT = Virginia Polytechnic Institute and State University, Blacksburg, Virginia, USA; UT = University of Tennessee, Texas, USA; OLE = Institute of Microbiology, Innsbruck, Austria.

Strain	Species	Location	Substrate	Date
CBS 101.448	*Omphalotus olivascens* var. *indigo*	Baja California, Mexico	*Quercus agrifolia*	14 Jan. 1995
CBS 101.447	*Omphalotus olivascens* var. *indigo*	Baja California, Mexico	*Quercus agrifolia*	1 Feb. 1995
VT 455	*Omphalotus olivascens* var. *olivascens*	California, USA	unknown	25 Nov. 1970
VT 1178	*Omphalotus olivascens* var. *olivascens*	California, USA	*Quercus agrifolia*	10 Feb. 1982
OLE 1	*Omphalotus olearius*	Prov. Parma, Italy	*Castanea sativa*	12 Oct. 1994
OLE 2	*Omphalotus olearius*	Prov. Parma, Italy	*Quercus sp.*	6 Oct. 1996
CBS 141.34	*Omphalotus illudens*	USA	unknown	1934
VT 0452	*Omphalotus illudens*	California, USA	unknown	10 Dec. 1971
VT 1946	*Omphalotus nidiformis*	Western Australia	on buried wood	10 June 1989
VT 1948	*Omphalotus nidiformis*	Western Australia	on dead *Banksia menziesii*	11 June 1989
VT 1949.01	*Omphalotus nidiformis*	Western Australia	*Acacia*	8 June 1989
VT 1490	*Omphalotus nidiformis*	Western Australia	unknown	13 July 1980
CBS 660.85	*Omphalotus subilludens*	Texas, USA	decaying wood stump	1967
CBS 101.446 (= UT 4866)	*Omphalotus mexicanus*	Guatemala	decaying wood stump	—
CBS 446.69	*Lampteromyces japonicus*	Japan	decaying *Fagus* sp.	March 1969
CBS 374.51	*Lampteromyces japonicus*	Japan	unknown	Feb. 1951

Omphalotus olearius (D.C.: Fr.) Sing. — Figs. 1–3

Omphalotus olearius (D.C.: Fr.) Sing., Lilloa 22 ('1949' 1951) 181.

Omphalotus illudens (Schwein.) Bresinsky & Besl — Figs. 4–6

Omphalotus illudens (Schwein.) Bresinsky & Besl, Beih. Sydowia 8 (1979) 106.

By many mycologists *O. illudens* is seen as conspecific with *O. olearius* (e.g. Watling & Gregory, 1989). A greenish staining of the pileipellis when treated with 25% ammonia solution or 30% KOH could be observed in all examined species of *Omphalotus*, a reaction easily recognisable when small fragments are squeezed between two glass slides. In contrast to other reports (e.g. Kuyper, 1995) refracting hyphae were found in the pileipellis of all *Omphalotus* species but they are rather scarce in most *O. olearius* collections. Thus, the only distinct morphological differences between *O. olearius* and *O. illudens* are the form and colour of the basidiomata: umbonate and uniformly yellowish orange coloured basidiomata are characteristic for *O. illudens*. A papilla like umbo was never observed in *O. olearius*; its pileus was always darker (usually reddish brown) than lamellae and stipe.

Pegler (1977), who considered *O. olearius* and *O. illudens* as one species, reported the occurrence of *O. olearius* in East Africa. His collection from Tanzania (K 50514; Figs. 10–11) consists of very small specimens: pilei about 2 cm in diameter, stipe 2 × 0.5 cm;

Table II. Morphological characters in *Omphalotus* and geographic distribution of taxa.

Characters	*O. olearius*	*O. illudens*	*O. subilludens*	*O. olivascens* var. *olivascens*	*O. olivascens* var. *indigo*	*O. nidiformis*	*O. mexicanus*
Distribution	southern Europe	USA, east coast, northern Europe?	southern USA, Mexico	USA, Texas, California	Mexico, Baja California	Australia, Tasmania	Mexico, Guatemala
Colours							
Pileus	orange to dark reddish brown	yellow orange	orange to dark reddish brown	dull orange to dark olive orange	olive orange,	whitish to blackish brown	bluish black to blackish
Lamellae	deep yellow, orange yellow	orange	orange, orange brown, reddish brown	olive to olive yellow	olive to olive yellow	whitish with a pink touch to yellowish orange	bluish black to blackish
Stipe	deep yellow, orange yellow	orange	deep yellow, orange yellow	olive to olive yellow, brick-red	olive to olive yellow, brick-red	whitish at the tip, brownish below	bluish black
Context (pileus, stipe)	deep yellow, orange yellow	deep yellow	deep yellow, orange yellow	dull orange olivaceous	bluish grey to blue violet	pure white	blue violet
Spores							
Length (L) (μm)	(5.0)6.4 ± 0.7 (8.0)	(4.6)5.0 ± 0.3 (5.7)	(6.3)7.0 ± 0.5 (8.1)	(6.2)7.4 ± 0.5 (8.8)	(6.7)7.9 ± 0.6 (9.7)	(5.0) 6.0 ± 0.6 (7.0)	(5.4) 6.0 ± 0.3 (6.7)
Width (W) (μm)	(4.6)5.7 ± 0.6 (7.6)	(4.0)4.7 ± 0.3 (5.2)	(4.2)4.8 ± 0.3 (5.8)	(5.8)6.9 ± 0.6 (8.2)	(6.0)7.4 ± 0.6 (9.0)	(4.6) 5.5 ± 0.6 (7.0) (7.0)	(4.6)5.2 ± 0.3 (5.8)
L/W	1.1 ± 0.1	1.1 ± 0.1	1.5 ± 0.2	1.1 ± 0.1	1.1 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Volume (μm^3)	(56)110 ± 32 (239)	(40)58 ± 8 (77)	(57) 86 ± 18 (145)	(112)184 ± 42 (295)	(128)232 ± 52 (412)	(55)96 ± 28 (187)	(69)85 ± 10 (104)
Pileipellis							
Refractive hyphae	rare	numerous	numerous	numerous	numerous	numerous	numerous
Incrusting pigment	orange to brownish	orange to brownish	orange to brownish	orange to brownish, violet	orange to brownish, violet	orange to brownish, violet	violet

Table III. Morphological characters in *Lampteromyces* and geographic distribution of taxa. Data are based on the original descriptions (Kawamura, 1915; Li & Hu, 1993; Zang, 1979).

Characters	*L. japonicus*	*L. mangensis*	*L. luminescens*
Distribution	Japan	Hunan, China	Xizang (Tibet), China
Colours			
Pileus	light brown, with yellowish or rosy tinges	white, sometimes with bluish tinges	white to pale purplish
Lamellae	white, sometimes with yellowish tinges	white to bluish violet	white
Stipe	light brown	white to pale bluish violet	white
Context (pileus, stipe)	white; dark purplish at the transition pileus -stipe	white, bluish violet the transition pileus -stipe	no data
Size			
Pileus		10–15 cm	5–9 cm
Flesh	1.3–2 cm		no data
Lamellae	9–18 mm	4–7(–11) mm	no data
Stipe	1.4–2.5 × 1.5–3 cm	2–3 × 2.5–3 cm	0.3–0.5 × 0.5–0.6 cm
Spores	smooth, 13–17 µm	smooth, 9.5–15 µm	rough, 12–14 µm
Cheilocystidia	none	none	fusiform
Gill edge	entire	entire	serrate
Annulus	present	present	absent

spore size (4.5) 5.2 ± 0.3 (5.8) × (4.2) 4.8 ± 0.3 (5.4) µm; quotient = 1.1 ± 0.1; volume = (41.8) 62.2 ± 12.0 (83.4) μm^3; pileipellis with numerous refractive hyphae; other elements incrusted with pale yellow pigment. These characters resemble those of *O. illudens*. The collection from Kenya (K 50515; Figs. 7–9) consists of one specimen only: it differs from *O. olearius* by its distinctly elliptic spores: (6.3) 6.9 ± 0.5 (7.9) × (3.3) 3.8 ± 0.3 (4.5) µm; quotient = 1.8 ± 0.1; volume = (36.4) 53.9 ± 11.1 (82.7) μm^3. The pileipellis is characterized by many hyphal tips with strongly thickened walls, some refractive hyphae, and few hyphal elements that are weakly incrusted with a pale yellow pigment. This set of features could not be found in any other investigated *Omphalotus* collection.

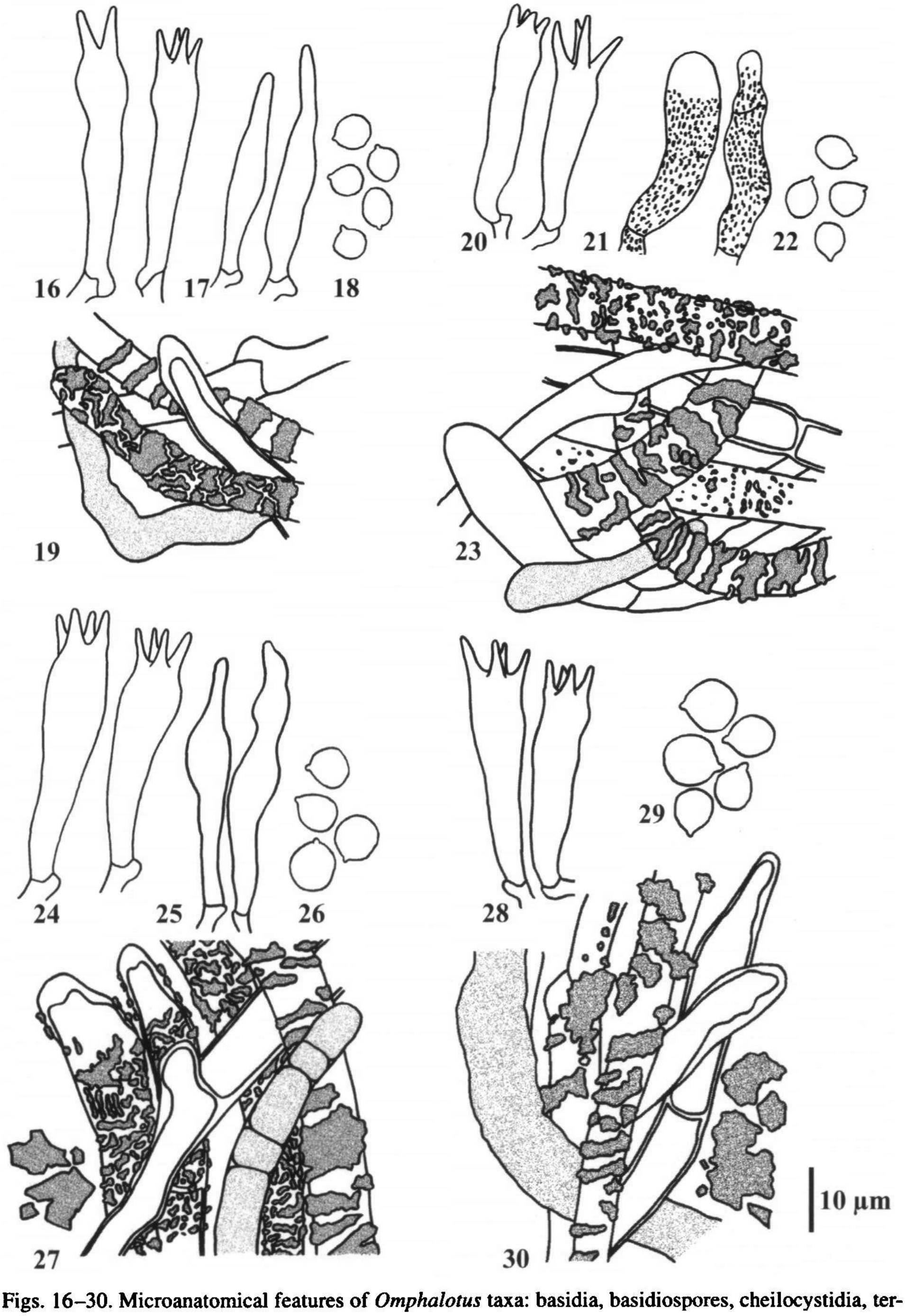
Omphalotus subilludens (Murrill) Bigelow — Figs. 12–15

Omphalotus subilludens (Murrill) Bigelow, Sydowia 35 (1982) 67.

This species is mainly characterized by its distinctly elliptic spores (quotient = 1.5 ± 0.2). The macroscopic characters are essentially the same as in *O. olearius* (Table II).

Omphalotus nidiformis (Berk.) O.K. Mill. — Figs. 16–19

Omphalotus nidiformis (Berk.) O.K. Mill., Mycol. Helv. 2 (1994) 93.



Figs. 16–30. Microanatomical features of *Omphalotus* taxa: basidia, basidiospores, cheilocystidia, terminal cells of the hymenophoral trama (21), and elements of the pileipellis. *Omphalotus nidiformis* (16–19), *O. mexicanus* (20–23), *O. olivascens* var. *olivascens* (24–27), and *O. olivascens* var. *indigo* (28–30). Refractive hyphae (light grey) and pigment incrustations (dark grey) are highlighted by shadings.

Populations of this taxon show at least two distinct colour forms: the colour of pileus ranges from white to brownish black. The context is always white (Miller, 1994). Size and shape of spores are of the *O. olearius* type (Table II).

Omphalotus mexicanus Guzmán & Mora — Figs. 20–23

Omphalotus mexicanus Guzmán & Mora, Bol. Soc. Mex. Mic. 18 (1983) 117.

Mora & Guzmán (1983) described amyloid elements in the gill trama (they called them 'pseudocystidia') and in the pileipellis. In our study amyloid elements could be found neither in the gill trama nor in the pileipellis. However, claviform to irregularly cylindrical terminal cells (Fig. 21) which are densely covered with very fine, dark violet crystal needles are rather frequent in the gill trama when observed in water. Also some hyphae in the pileipellis are incrusted with such crystals. This striking pigment dissolves in 2.5% KOH (the medium stains greenish) but remains unchanged in Melzer's reagent.

Omphalotus olivascens Bigelow, O.K. Mill. & Thiers var. **olivascens** & **Omphalotus olivascens** var. **indigo** Moreno, Esteve-Rav., Pöder & Ayala — Figs. 24–30

Omphalotus olivascens Bigelow, O.K. Mill. & Thiers var. *olivascens*, Mycotaxon 3 (1976) 363.
Omphalotus olivascens var. *indigo* Moreno, Esteve-Rav., Pöder & Ayala, Mycotaxon 48 (1993) 218.

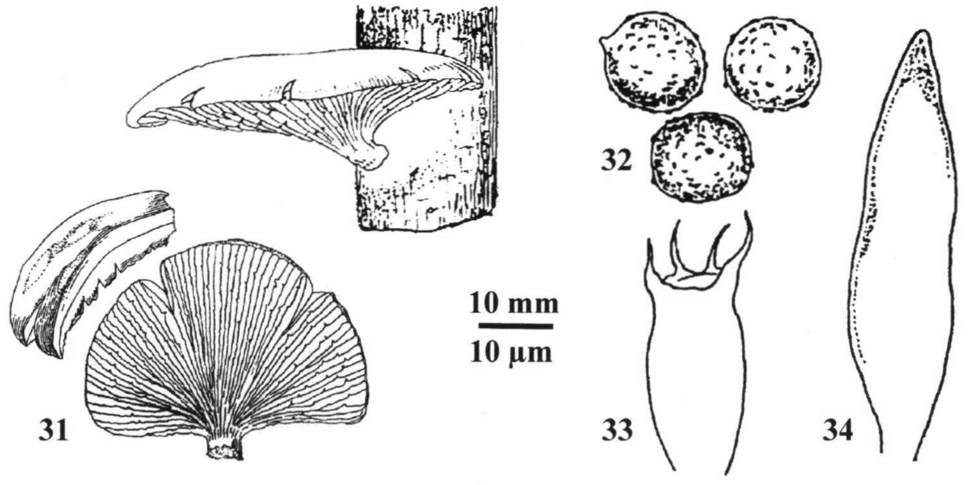
The two varieties of *O. olivascens* are distinguished from other species of *Omphalotus* by distinctive olive overtones in all parts of their basidiomata. The spores are usually larger as in other species of the genus (Table II), although Bigelow et al. (1976) mentioned that "some specimens have some smaller spores (5–7 µm) which lead to ranges which overlap with those of the other species". *Omphalotus olivascens* var. *indigo* shows in general the same features as the type variety but differs in its distinctly bluish grey to blue violet context (Moreno et al., 1993).

Lampteromyces Sing.

Lampteromyces Sing., Mycologia 39 (1947) 79–80.

The genus was originally based on one species: *L. japonicus* (Kawam.) Sing. (Singer, 1947). Singer (1986) delimited this genus from *Omphalotus* by its voluminous spores (up to 17 µm in diameter) and the presence of a veil. Chemical characters such as the occurrence of *Boletales* pigments and of sesquiterpenes of the illudane type are very similar in *L. japonicus* and in *Omphalotus* species (Kämmerer et al. 1985). Until now two further species in the genus *Lampteromyces* have been described: *L. luminescens* M. Zang and *L. mangensis* J. Li & X. Hu.

Lampteromyces luminescens is described to have no veil, punctate spores, fusiform cheilocystidia, serrate gill edges and, compared to the other two species, a relative slender stipe (Zang, 1979). For more characters see Table III and Figs. 31–34. Microchemical reactions (e.g. amyloidity of spores) are not mentioned in the original description. Although no material was available for our studies it seems likely that *Lampteromyces luminescens* belongs to *Lentinellus*.



Figs. 31–34. *Lampteromyces luminescens*: basidiomata (31), basidiospores (32), basidium (33), cheilocystidium (34); after Zang (1979, 1984).

Lampteromyces mangensis differs from *L. japonicus* mainly in its more or less whitish colours. The description of this species sounds very similar to Corner's diagnoses of the Malaysian *Pleurotus olivascens* Corner (Corner, 1981), a species already discussed to be included in *Lampteromyces* by Singer (1986). No authentic material of *L. mangensis* and *P. olivascens* could be studied by us.

THIN LAYER CHROMATOGRAPHY OF PIGMENTS

Pigments from dried material

With the exception of *O. mexicanus* a very similar pigment pattern could be observed for all species (Fig. 35, Table IV). All species were characterized by the presence of atromentin and thelephoric acid although these pigments were produced in quite different quantities: In the two varieties of *O. olivascens* very large amounts of atromentin could be detected whereas in *O. olearius* and *O. nidiformis* only traces of this pigment were found. Medium quantities of atromentin were produced by *O. illudens*, *O. mexicanus*, and *O. subilludens*. Thelephoric acid was abundant in all species except in *O. olearius* and *O. subilludens* where only small amounts were detectable.

Pigments from cultures

A survey of the known Boletales pigments in *Omphalotus* and *Lampteromyces* is given in Table IV. Atromentin and thelephoric acid were found in all culture extracts. Variegatic acid, although not detectable in dried material, was present in *Lampteromyces japonicus* and all *Omphalotus* strains. The red pigment variegatorubin, found in all culture extracts, might be an artefact caused by oxidation during extraction (Gill & Steglich, 1987). The presence of xerocomic acid could be shown in cultures of *O. illudens*, *O. mexicanus* and the two varieties of *O. olivascens*. The cyclopentanoid 'gyroporin' and the pulvinic acid derivate 'atromentinic acid', which had been found by Bresinsky & Besl (1979b) in *O. illudens*, *O. olearius* and *L. japonicus* were not clearly detectable in this study. Moreover, a number of unidentified pigments was found in all strains but the individual patterns did not indicate substantial differences between species because the amounts of produced pigments varied strongly within different strains or collections of one species and, consequently, weak bands of pigments might have been masked (Fig. 36, Table V).

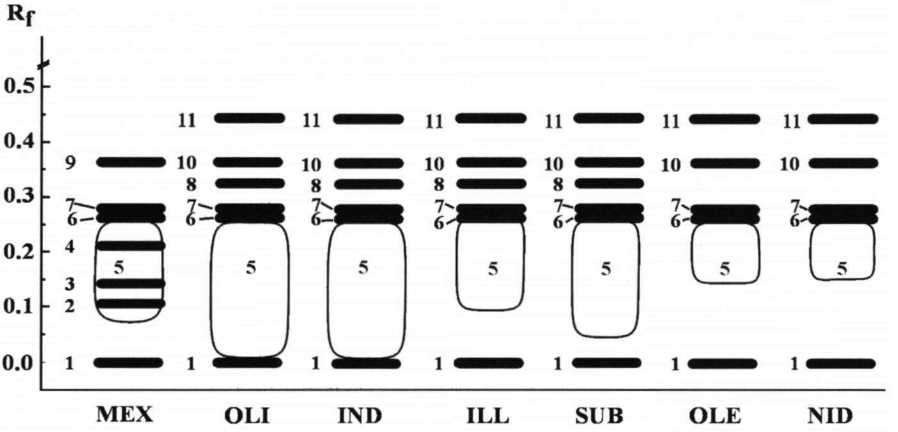


Fig. 35. Schematic representation compiling TLC pigment patterns from dried material. Acronymes: MEX = *O. mexicanus*; OLI = *O. olivascens* var. *olivascens*; IND = *O. olivascens* var. *indigo*; ILL = *O. illudens*; SUB = *O. subilludens*; OLE = *O. olearius*; NID = *O. nidiformis*. The different pigment bands (indicated by numbers) are characterised in Table V.

Table IV. Pigments in *Omphalotus* and *Lampteromyces* (C = from cultures; D = from dried material; F = from fresh basidiomata). Numerical indices refer to the initial detection of pigments: (1) Kämmerer et al., 1985; (2) Sullivan et al., 1971; (3) Sullivan & Guess, 1969; (4) Bresinsky & Besl, 1979b; (5) Singh & Anchel, 1971; (4) Bresinsky & Besl, 1979a.

	O. illudens	*O. olearius*	*O. olivascens* var. *olivascens*	*O. olivascens* var. *indigo*	*O. mexicanus*	*O. nidiformis*	*O. subilludens*	*L. japonicus*
Atromentin	C, D	C(1), D	C, D	C, D	C, D	C, D	C(2), F(3)	C(1), D
Atromentinic acid	C(4), F(5)	C(4)	–	–	–	–	–	C(1)
Gyroporin	C(6)	C(4)	–	–	–	–	–	C(1)
Thelephoric acid	C, D, F(6)	C, D	C, D	C, D	C, D	C, D	C(2), D	C(1)
Variegatic acid	C	C(4)	C	C	C	C	C	C
Variegatorubin	C	C	C	C	C	C	C	C
Xerocomic acid	C	C(4)	C	C	C	–	–	–

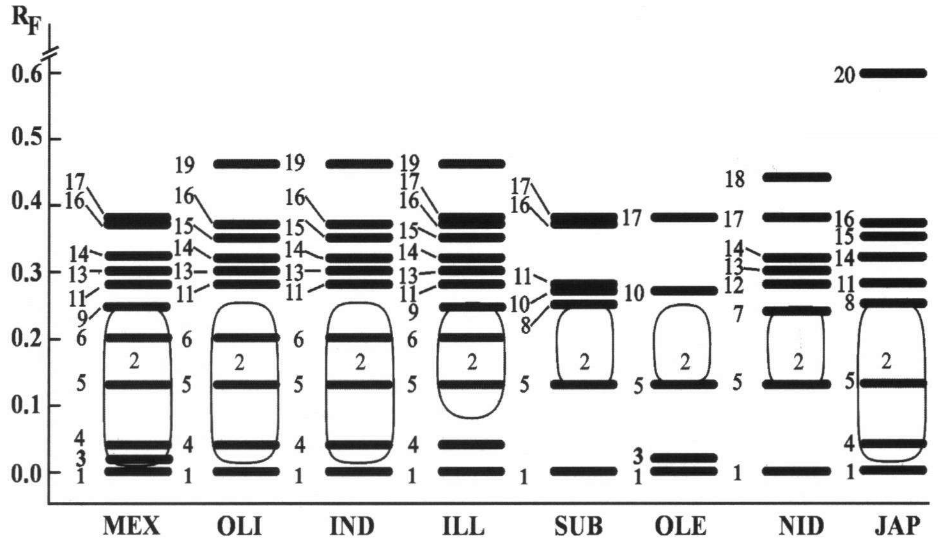


Fig. 36. Schematic representation compiling TLC pigment patterns from culture extracts. Acronymes: MEX = *O. mexicanus*; OLI = *O. olivascens* var. *olivascens*; IND = *O. olivascens* var. *indigo*; ILL = *O. illudens*; SUB = *O. subilludens*; OLE = *O. olearius*; NID = *O. nidiformis*; JAP = *Lampteromyces japonicus*. The different pigment bands (indicated by numbers) are characterised in Table VI.

Table V. Characterisation of the bands shown in Fig. 35 (pigment patterns from extracts of dried material).

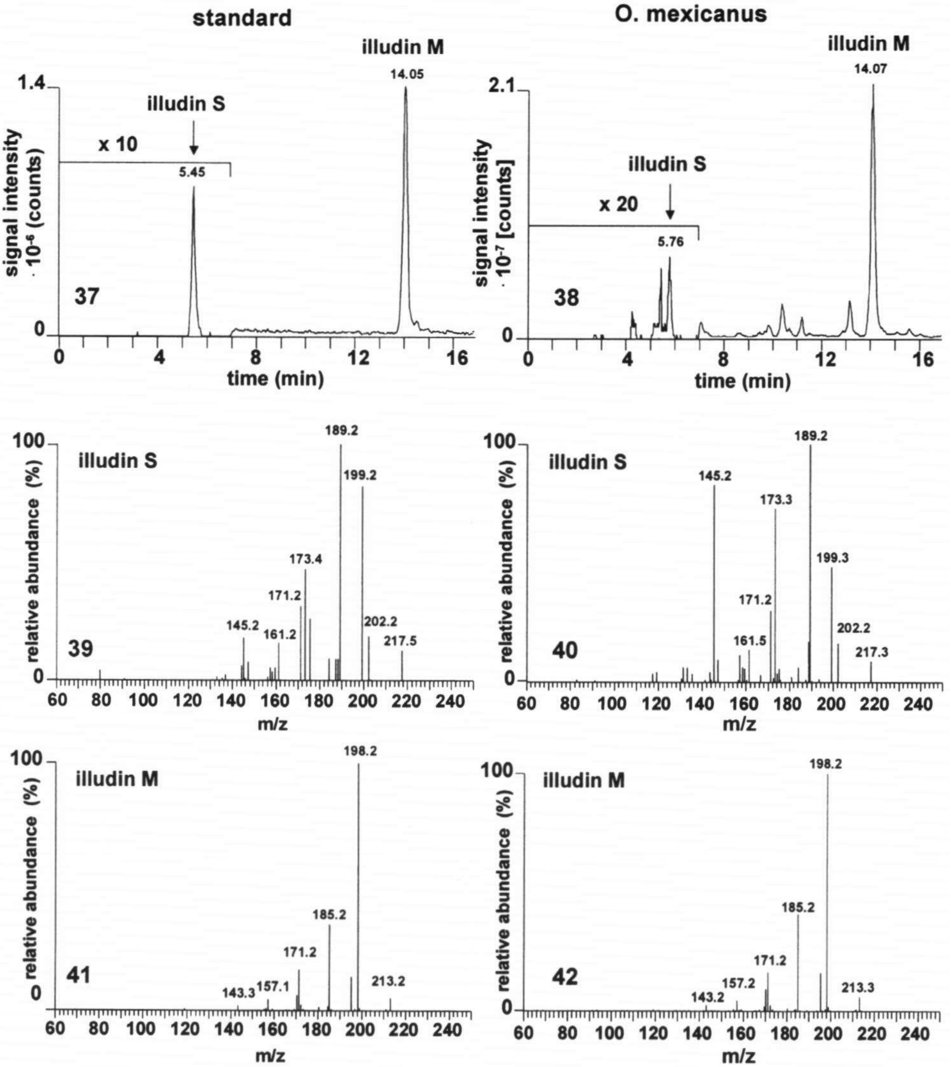
Nr.	Rf-value	daylight	UV 366 nm	Species
1	0	–	–	starting point
2	0.10	yellow	dark	*O. mexicanus*
3	0.15	turquoise	dark	*O. mexicanus*
4	0.22	red	yellow	*O. mexicanus*
5	0–0.25 (atromentin)	greenish	dark	*O. mexicanus, O. olivascens* var. *olivascens,* var. *indigo, O. olearius, O. nidiformis, O. illudens, O. subilludens*
6	0.27	yellow	yellow	*O. mexicanus, O. olivascens var. olivascens,* var. *indigo, O. olearius, O. nidiformis, O. illudens, O. subilludens*
7	0.28	orange	yellow	*O. mexicanus, O. olivascens* var. *olivascens,* var. *indigo, O. olearius, O. nidiformis, O. subilludens*
8	0.33	yellow	dark	*O. olivascens* var. *olivascens,* var. *indigo, O. illudens, O. subilludens*
9	0.37	turquoise	blue	*O. mexicanus*
10	0.37	yellow	blue	*O. olivascens* var. *olivascens,* var. *indigo, O. olearius, O. nidiformis, O. illudens, O. subilludens*
11	0.46	yellow	orange	*O. olivascens* var. *olivascens,* var. *indigo, O. olearius, O. nidiformis, O. illudens, O. subilludens*

Table VI. Characterisation of the bands shown in Fig. 36 (pigment patterns from culture extracts).

Nr.	R_f	daylight	UV 366 nm	Pigment	Species
1	0	–	–	–	Starting point
2	0 – 0.25	greenish	dark	atromentin	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *O. subilludens*, *O. olearius*, *O. nidiformis*, *O. mexicanus*, *L. japonicus*
3	0.02	red	dark	unknown	*O. olearius*, *O. mexicanus*
4	0.04	yellow	dark	unknown	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *O. mexicanus*, *L. japonicus*
5	0.13	yellow	dull yellow	variegatic acid	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *O. subilludens*, *O. olearius*, *O. nidiformis*, *O. mexicanus*, *L. japonicus*
6	0.20	yellow	yellow	xerocomic acid	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *O. mexicanus*
7	0.24	yellow	yellow	unknown	*O. nidiformis*
8	0.25	red	dark	unknown	*O. subilludens*, *L. japonicus*
9	0.26	yellow	dark	gyroporin?	*O. illudens*, *O. mexicanus*
10	0.27	blue	dark	unknown	*O. subilludens*, *O. olearius*
11	0.28	yellow	dark	unknown	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *O. subilludens*, *O. mexicanus*, *L. japonicus*
12	0.28	red	orange	unknown	*O. nidiformis*
13	0.30	yellow	yellow	unknown	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *O. nidiformis*, *O. mexicanus*
14	0.32	red	dark	variegato-rubin	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *O. nidiformis*, *O. mexicanus*, *L. japonicus*
15	0.35	yellow	dark	unknown	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *L. japonicus*
16	0.37	red	dark	unknown	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*, *O. subilludens*, *O. mexicanus*, *L. japonicus*
17	0.38	yellow	dark		*O. illudens*, *O. subilludens*, *O. olearius*, *O. nidiformis*, *O. mexicanus*
18	0.44	yellow	dark	unknown	*O. nidiformis*
19	0.46	yellow	dark	unknown	*O. olivascens* var. *olivascens*, var. *indigo*, *O. illudens*
20	0.59	yellow	dark	unknown	*L. japonicus*

DETECTION OF ILLUDIN S AND ILLUDIN M

Earlier studies reported the occurrence of the sesquiterpenes illudin S and illudin M in *Lampteromyces japonicus* (Nakanishi et al., 1965) and in many *Omphalotus* species (Anchel et al., 1952; Nair et al., 1983; Kirchmair et al., 1999). In this study *O. mexicanus* was shown to produce both illudin S and illudin M. The chromatographic retention times of illudin S and illudin M were obtained by injection of reference solutions in acetonitrile. Using a 150



Figs. 37–42. Detection and identification of illudins in an ethyl acetate extract of *O. mexicanus* by high-performance liquid chromatography-mass spectrometry. For chromatographic and mass spectrometric conditions refer to method section; 37, 38. reconstructed ion chromatograms detecting the transitions 247.1→217.1→full scan from 60-250 u and 231.1→213.2→full scan from 60-250 u, specific for illudin S and M; 39 & 40. MS^3 spectra of the illudin S reference standard and illudin S in *O. mexicanus*, respectively; 41, 42. MS^3 spectra of the illudin M reference standard and illudin M in *O. mexicanus*, respectively.

× 2 mm I.D. column packed with a Nucleosil ODS stationary phase and applying a gradient of 10–42% acetonitrile in 0.1% aqueous acetic acid in 20 min, illudin S eluted first at 5.4 min and illudin M at 14.1 min (Figs. 37, 38). Analysis of the MS^3 spectra under the peaks at 5.4 min and 14.1 min of the *O. mexicanus* extract revealed fragment ions characteristic for illudin S and illudin M, respectively (Figs. 39–42). The high specificity of chromatographic separation in combination with MS^3 and the congruence of the obtained mass spectra with those of the reference standards prove the presence of both illudin S and illudin M in *O. mexicanus*.

DISCUSSION

Dried material is useful for morphological research but many characteristic pigments are rapidly degraded. Therefore, herbarium specimens may be useful for chemotaxonomical fingerprints but not for the identification of group characteristic metabolites. For extensive comparative chemotaxonomical studies fresh basidiomata are usually not available. Thus, culture extracts provide a suitable alternative. But it should be mentioned that the pigment pattern in native basidiomata need not be identical to that in mycelium cultures (Bresinsky, 1974).

In all *Omphalotus* and *Lampteromyces* species typical Boletales pigments of the pulvinic acid type as well as sesquiterpenes of the illudane type were found. As already discussed by Kämmerer et al. (1985) the combination of these two features is highly characteristic for *Omphalotus* and *Lampteromyces* and cannot be found in any other mushroom group. Because of the uniqueness of the metabolites illudin S and illudin M Nair et al. (1983) proposed the placement of *Lampteromyces* in *Omphalotus*. Kämmerer et al. (1985) stressed that in contrast to the more irregular hymenophoral trama in *Lampteromyces*, *Omphalotus* has a distinctly divergent gill anatomy. In the present study a distinctly divergent tramal structure could not be observed in *Omphalotus* (see also Singer, 1986). Thus, the large spores and the occurrence of a veil in *Lampteromyces* remain as reliable distinguishing characters. In other genera, e.g. in *Armillaria* or in *Pleurotus*, species with and without veil have been described. Moreover, sequence analyses of the ribosomal ITS1-5.8S-ITS2 region demonstrated that *L. japonicus* clustered within the clade of *Omphalotus* and was not assigned to a separate clade (Stolz, 1999). So it seems logical to regard *Lampteromyces* as a synonym of *Omphalotus*. The following new combinations are necessary:

Omphalotus japonicus (Kawam.) Kirchm. & O.K. Mill., *comb. nov.*

Basionym: *Pleurotus japonicus* S. Kawamura, Journ. Coll. Sci. Imp. Univ. Tokyo 35 (3) (1915) 2.

Omphalotus mangensis (J. Li & X. Hu) Kirchm. & O.K. Mill., *comb. nov.*

Basionym: *Lampteromyces mangensis* J. Li & X. Hu, Acta Sci. Nat. Univ. Norm. Hunan 16 (1993) 188.

Lampteromyces luminescens M. Zang differs in macro- and microscopical characters from *Omphalotus* species. Luminosity of the basidiomata is the only character it shares with *Omphalotus* species. Its rough spores, fusiform cheilocystidia and the serrate gill edges make an affiliation to *Lentinellus* probable.

In literature, the delimitation of the different *Omphalotus* species is controversial. *Omphalotus mexicanus* and *O. nidiformis*, for example, can be accepted as good morphospecies on account of their unique characters (Table II): the entire basidioma of the former is bluish black, and terminal cells covered with violet crystal needles can be found in its hymenophoral trama. White lamellae and a white context are typical for *O. nidiformis* (Miller, 1994), features it shares with *O. japonicus* and *O. mangensis*. However, the latter two species are characterized by an annular zone at the stipe apex and much bigger spores. The opinion that *O. mexicanus* and *O. nidiformis* are separate species is supported by the findings of Petersen & Hughes (1998) who reported only a low sexual compatibility of *O. nidiformis* with other *Omphalotus* species. The results of their RFLP analyses of the ribosomal ITS1-5.8S-ITS2 region confirmed the relatively isolated position of these two taxa (Hughes & Petersen, 1998).

There has long been disagreement whether or not the American *O. illudens* and the European *O. olearius* are two different species. Mating experiments affirmed that *O. illudens* ranks as species from North America (Petersen & Hughes, 1998). But it remains still unclear if beside *O. olearius* also *O. illudens* exists in Europe since there are only few morphological separating characters: the colour of *O. illudens* is paler (uniformly yellowish orange), the pileus is umbonate and refractive hyphae are abundant in the pileipellis. Using these criteria it seems that the collections from Belgium belong to *O. illudens* while all collections from the mediterranean region can be recognised as *O. olearius*.

Omphalotus subilludens is morphological very similar to *O. olearius* but differs in its elliptic spores and numerous refractive hyphae in the pileipellis. *Omphalotus olivascens* is distinguished from *O. olearius* mainly by its more olive colours (the context is blue in its variety *indigo*), the abundant refractive hyphae, and significantly bigger spores. The olive and blue colours are due to the very high amount of the pigment atromentin (Kirchmair & Griesser, 1999). Petersen & Hughes (1998) noticed a very high level of sexual intercompatibility between collections of the three taxa mentioned before. Moreover, Hughes & Petersen (1998) found no differences in the RFLP patterns between *O. olearius* and *O. subilludens*. *Omphalotus olivascens* differed in one restriction site from the two former taxa. The findings of these authors indicate that these three taxa belong to one biological species. Nevertheless, since they are geographically well isolated and differ at least in two morphological characters, they can be considered as different morphospecies in agreement with a morphological species concept (see Kuyper, 1988).

KEY TO THE SPECIES OF OMPHALOTUS

1a. Basidioma annulate 2
b. Basidioma not annulate 3
2a. Entire basidioma more or less white .... *O. mangensis*
b. Basidioma brownish .... *O. japonicus*
3a. Context in pileus and stipe ± white .... *O. nidiformis*
b. Context coloured 4
4a. Spores elliptical (quotient = 1.5 ± 0.2) .... *O. subilludens*
b. Spores spherical (quotient = 1.1 ± 0.1) 5
5a. Basidioma somewhere with olive tinges 6
b. Basidioma without olive tinges 7
6a. Context olivaceous, not blue .... *O. olivascens* var. *olivascens*
b. Context distinctly blue, bluish grey .... *O. olivascens* var. *indigo*
7a. Entire basidioma dark blue to bluish black .... *O. mexicanus*
b. Basidioma not so 8
8a. Basidioma uniformly yellow orange, pileus umbonate, pileipellis with numerous refractive hyphae .... *O. illudens*
b. Pileus usually ± orange brown, darker than the lamellae, infundibuliform, refractive hyphae usually rare .... *O. olearius*

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REFERENCES

Anchel, M., A. Hervey & W.J. Robbins. 1952. Production of illudin M and a fourth crystalline compound by Clitocybe illudens. Proc. Nat. Acad. USA: 28: 927–928.

Bigelow, H.E. 1982. Species described in Clitocybe by C.H. Peck and W.A. Murrill. Sydowia 35: 37–74.

Bigelow, H.E., O.K. Miller Jr. & H.D. Thiers. 1976. A new species of Omphalotus. Mycotaxon 3: 363–372.

Bresinsky, A. 1974. Zur Frage der taxonomischen Relevanz chemischer Merkmale bei höheren Pilzen. Bull. Soc. Linn. Lyon, numéro spécial 1974: 61–84.

Bresinsky, A. & H. Besl. 1979a. Notizen über Vorkommen und systematische Bewertung von Pigmenten in höheren Pilzen (3) – Untersuchungen an Boletales aus Amerika. Z. Mykol. 45: 247–264.

Bresinsky, A. & H. Besl. 1979b. Zum verwandtschaftlichen Anschluß von Omphalotus. Beih. Sydowia 8: 98–109.

Bresinsky, A. & P. Orendi. 1970. Chromatographische Analyse von Farbmerkmalen der Boletales und anderer Makromyceten auf Dünnschichten. Z. Pilzk. 36: 135–169.

Bresinsky, A. & A. Rennschmied. 1971. Pigmentmerkmale und Organisationsstufen und systematische Gruppen bei höheren Pilzen. Ber. dt. bot. Ges. 84: 313–329.

Corner, E.J.H. 1981. The agaric genera Lentinus, Panus and Pleurotus with particular reference to Malaysian species. Beih. Nova Hedwigia 69: 1–169.

Gill, M. & W. Steglich. 1987. Pigments of Fungi (Macromycetes). Progr. Chem. Org. Nat. Prod. 51: 1–317

Hughes, K.W. & R.H. Petersen. 1998. Relationships among Omphalotus species (Paxillaceae) based on restriction sites in the ribosomal ITS1-5.8S-ITS2 region. Pl. Syst. Evol. 211: 231–237.

Kämmerer, A., H. Besl & A. Bresinsky. 1985. Omphalotaceae fam. nov. und Paxillaceae, ein chemotaxonomischer Vergleich zweier Pilzfamilien der Boletales. Pl. Syst. Evol. 150: 101–117.

Kawamura, S. 1915. Studies on the Luminous Fungus Pleurotus japonicus sp. nov. J. Coll. Sci. imp. Univ. Tokyo 35: 1–29.

Kirchmair, M. & U.J. Griesser. 1999. In-situ-detection of atromentin in Omphalotus olivascens var. indigo by FTIR-microspectroscopy. XIII Congress of European mycologists, book of abstracts: 21–25 September 1999: 65. Alcalá de Henares (Madrid) Spain.

Kirchmair, M., R. Pöder & C.G. Huber. 1999. Identification of illudins in Omphalotus nidiformis and Omphalotus olivascens var. indigo by column liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry. J. Chromatogr. A 832: 247–252.

Kögl, F. & J.J. Postowsky. 1924. Untersuchungen über Pilzfarbstoffe. I. Über das Atromentin. Liebigs Ann. Chem. 440: 19–35.

Kuyper, Th.W. 1988. Specific and infraspecific delimitation. In: C. Bas et al. (Eds.), Flora agaricina neerlandica 1: 30–37. Balkema, Rotterdam & Brookfield.

Kuyper, Th.W. 1995. Omphalotus Fay. In: C. Bas et al. (Eds.), Flora agaricina neerlandica 3: 88–99. Balkema, Rotterdam & Brookfield.

Li, J. & X. Hu. 1993. A new species of Lampteromyces from Hunan. Acta. Sci. Nat. Univ. Norm. Hunan 16: 188–189.

Miller Jr., O.K. 1994. Observations on the genus Omphalotus in Australia. Mycol. Helv. 2: 91–100.

Mora, V. & G. Guzmán. 1983. Agaricales poco conogidos en estado de Morelos. Bol. Soc. Mex. Mic. 18: 115–139.

Moreno, G., F. Esteve-Raventós, R. Pöder & N. Ayala. 1993. Omphalotus olivascens var. indigo, var. nov. from Baja California (Mexico). Mycotaxon 48: 217–222.

Nair, M.S.R., S.T. Carey & C.T. Rogerson. 1983. Illuoids from Omphalotus olivascens and Clitocybe subilludens. Mycologia 75: 920–922.

Nakanishi, K., M. Ohashi, M. Tada & Y. Yamada. 1965. Illudin S (lampterol). Tetrahedron 21: 1231–1246.

Pegler, D.N. 1977. A preliminary agaric flora of East Africa. Kew Bull. Add Ser. 6: 1–615.

Petersen, R.H. & K.W. Hughes. 1998. Mating types in Omphalotus (Paxillaceae, Agaricales). Pl. Syst. Evol. 211: 217–229.

Singer, R. 1947. New genera of fungi. III. Mycologia 39: 77–89.

Singer, R. 1949 (publ. 1951). The Agaricales (Mushrooms) in modern taxonomy. Lilloa 22: 1–832.

Singer, R. 1986. The Agaricales in modern taxonomy. Ed. 4. Koenigstein.

Singh, P. & M. Anchel. 1971. Atromentic acid from Clitocybe illudens. Phytochemistry 10: 3259–3262.

Stolz, D. 1999. Zur Taxonomie der Gattung Omphalotus (Basidiomycetes): Seuenzanalysen ribosomaler DNA (ITS). Master's thesis (unpubl.), Univ. Innsbruck, Institute of Microbiology & Institute of Zoology and Limnology.

Sullivan, G., R.D. Garrett & R.F. Lenehan. 1971. Occurrence of Atromentin and Thelephoric Acid in Cultures of Clitocybe subilludens. J. Pharm. Sci.: 1727–1729.

Sullivan, G. & W.L. Guess. 1969. Atromentin: A smooth muscle stimulant in Clitocybe subilludens. Lloydia 32: 72–75.

Watling, R. & N.N. Gregory. 1989. British Fungus Flora 6/Crepidotaceae, Pleurotaceae and other pleurotoid agarics. Edinburgh.

Zang, M. 1979. Some new species of Higher Fungi from Xizang (Tibet) of China. Acta Bot. Yunn. 1: 101–105.

Zang, M. 1984. Mushroom distribution and diversity of habitats in Tibet, China. McIlvainea 6: 14–20.