Presence of *Pleurotus ostreatus* in Patagonia, Argentina

Bernardo Ernesto Lechner[1], Ronald Petersen[2], Mario Rajchenberg[3] & Edgardo Albertó[4]

[1]PRHIDEB-CONICET, Departamento de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, Argentina; [2]Department of Botany, University of Tennessee, Knoxville, Tennessee, USA; [3]CIEFAP, Centro de Investigación y Extensión Forestal Andino Patagónico. Esquel, Chubut, Argentina; [4]Instituto de Investigaciones Biotecnológicas IIB-INTECH (UNSAM-CONICET), Chascomús, Buenos Aires, Argentina

Summary

Specimens belonging to the genus *Pleurotus* were collected growing on fallen trunks of *Araucaria araucana*, a native tree with a poorly known mycoflora, which grows in Patagonia, Argentina. Fruitbodies were produced in culture on sawdust from an isolated strain. Interspecific pairing tests performed between mating types of *Pleurotus* from Patagonia and tester strains of *P. pulmonarius* and *P. ostreatus* showed the Patagonia strain to be 100 % compatible with *P. ostreatus* and incompatible with *P. pulmonarius*. Dikaryons obtained on sawdust were fertile, since they were able to produce fruitbodies and viable spores. This is the first documented record of *P. ostreatus* from Argentina and the first gilled fungus found growing on *Araucaria araucana*.

Key words *Pleurotus ostreatus*, *Araucaria araucana*, Mating tests, Patagonia

Presencia de *Pleurotus ostreatus* en Patagonia, Argentina

Resumen

Fueron recolectados specímenes del género *Pleurotus* que crecían sobre troncos de *Araucaria araucana*, un árbol nativo de la Patagonia argentina del que se conocen muy pocos hongos que se desarrollan sobre él. Las fructicaciones fueron obtenidas a partir de una cepa empleando aserrín. Se realizaron varios ensayos de compatibilidad interpespecífica entre los polos de compatibilidad sexual del *Pleurotus* de la Patagonia y cepas control de *Pleurotus pulmonarius* y *Pleurotus ostreatus*. Los resultados indicaron que las cepas de la Patagonia fueron 100 % compatibles con *P. ostreatus* e incompatibles con *P. pulmonarius*. Los dicariontes obtenidos a partir de los apareamientos produjeron fructificaciones fértiles ya que los basidiocarpos produjeron esporas viables. Este es la primera cita documentada para esta especie en la Argentina y el primer hongo con laminillas encontrado creciendo sobre *Araucaria araucana.*

Palabras clave *Pleurotus ostreatus, Araucaria araucana*, Ensayos de compatibilidad sexual, Patagonia

Knowledge of the genus *Pleurotus* in Argentina is scant and only few species have been recorded, namely *Pleurotus eryngii* (DC.) Gill., *Pleurotus laciniatocrenatus* Speg., *Pleurotus ostreatus* (Jacq.: Fr.) Kummer, *Pleurotus pulmonarius* (Fr.: Fr.) Quél., *Pleurotus rickii* Bres., *Pleurotus smithii* Guzman, and *P. sutherlandi* Singer [1-10]. For the Patagonian Andean forest in southern Argentina only *P. eryngii* and *P. sutherlandi* have been recorded [4]. The latter species is *Panellus longinquus* (Berk.) Singer, according to Horak [11].

In recent years we have collected several specimens of a *Pleurotus* growing on *Araucaria araucana* K. Koch, a native tree species distributed in the northern area of Patagonia. This fungus is very interesting since only few species of Hyphomycetes and Basidiomycetes have been recorded growing on *Araucaria* [12,13]. In this paper we describe and illustrate this fungus, and report interspecific mating tests in order to determine its taxonomic position.

Dirección para correspondencia:
Dr. E. Albertó
Instituto de Investigaciones Biotecnológicas IIB-INTECH (UNSAM-CONICET),
Camino de Circ., Km 6,
(7130) Chascomús,
Buenos Aires, Argentina
E-mail: ealberto@intech.gov.ar

Aceptado para publicación el 15 de Marzo de 2002

MATERIALS AND METHODS

Macro- and micromorphological description. Specimens were described based on characters observed in naturally occurring specimens and in specimens obtained in culture. Color terms and annotations follow Munsell [14]. Abbreviations of author's names are according to Kirk & Ansell [15]. Freehand sections of specimens were mounted in 5% KOH plus 1% aqueous solution of phloxine and in Melzer's reagent. All collections are deposited in the Herbarium of Mario Rajchenberg (MR) at CIEFAP and in the Mycology Herbarium, Dept. de Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, University of Buenos Aires (BAFC). Herbaria abbreviations follow Holmgren *et al.* [16].

Mating studies. Monosporic cultures were obtained from water dilutions of fresh spore prints on sterile aluminum paper; spores were suspended in 10 ml of sterile-distilled water containing 0.01 ml Tween 80 to avoid agglutination. This solution was diluted to 1/10, 1/100 and 1/1000. Petri dishes containing Nobles' medium [17] were inoculated with 1 ml of each of these dilutions and incubated in the dark at 25 °C. Germlines were reisolated into slanted tubes, discarding all those having clamps. Cultures obtained were deposited in the mycological culture collection of the IIB-INTECH. Haplonts were confronted in pairs in Petri dishes using 7-mm diameter blocks as inoculum. Plates were incubated in the dark at 25 °C, and after one week hyphae were observed under the microscope both in the contact zone and in the lateral area [18]. A positive result (+) was reckoned by the presence of clamps, and a negative one (-) by their absence. Self-crossing tests were done in order to obtain mating types. Interspecific pairings were later carried out using the test strains of *Pleurotus ostreatus* (OSTR) 3505 and *P. pulmonarius* (PULM) 4203 from the culture collection of the Department of Botany, University of Tennessee, Knoxville (USA).

Basidiome production. Traditional methods for fruiting *Pleurotus* species were used [19,20]. A mixture of sawdust of willow, *Salix* sp., (70%), wheat meal (10%), oatmeal (4%) and $CaCO_3$ (1%) was introduced in polypropylene bags and autoclaved at 120 °C for 2 h. After cooling they were inoculated with strain BAFC 120 obtained from specimens MR 10813, and incubated in the dark at 25 °C. After 15 days, bags were kept at 18-20 °C with 9 h light/ 15 h darkness photoperiod to induce basidiome formation.

Material studied. Argentina, Neuquén, Aluminé, Moquehue, leg. Jorge del Vas, III/93 MR 10813 (BAFC 50119); Lanín National Park, sectional Tromen, growing on *Araucaria araucana*, leg. M. Rajchenberg, 20/V/1999, MR N ° 11940, 11941 and 11942. Basidiomata obtained in culture, Llavallol, leg. B. Lechner & E. Albertó, IV/1999, BAFC 50563.

RESULTS

Description of basidiomata studied. Pileus 65-110 x 80-130 mm, spathulate to flabelliform (Figure 1), light yellowish brown (Munsell 10YR 6/4), smooth, glabrous; margin entire, inrolled when young, more or less straight at maturity. Lamellae decurrent, not forming a reticulum on the stipe, crowded, width about 5 mm, pale whitish (Munsell 2,5Y 8/4) becoming lemon yellow on drying; lamella edge entire. Stipe 15-20 x 11-15 mm, eccentric to lateral, whitish to cream coloured, short, slightly smooth to longitudinally striate in upper part, solid, in clusters. Flesh white, more or less thin (2-6 mm), fragile when fresh with a coriaceous texture in dry specimens. Odour pleasant, taste mild. Spore print white to cream coloured.

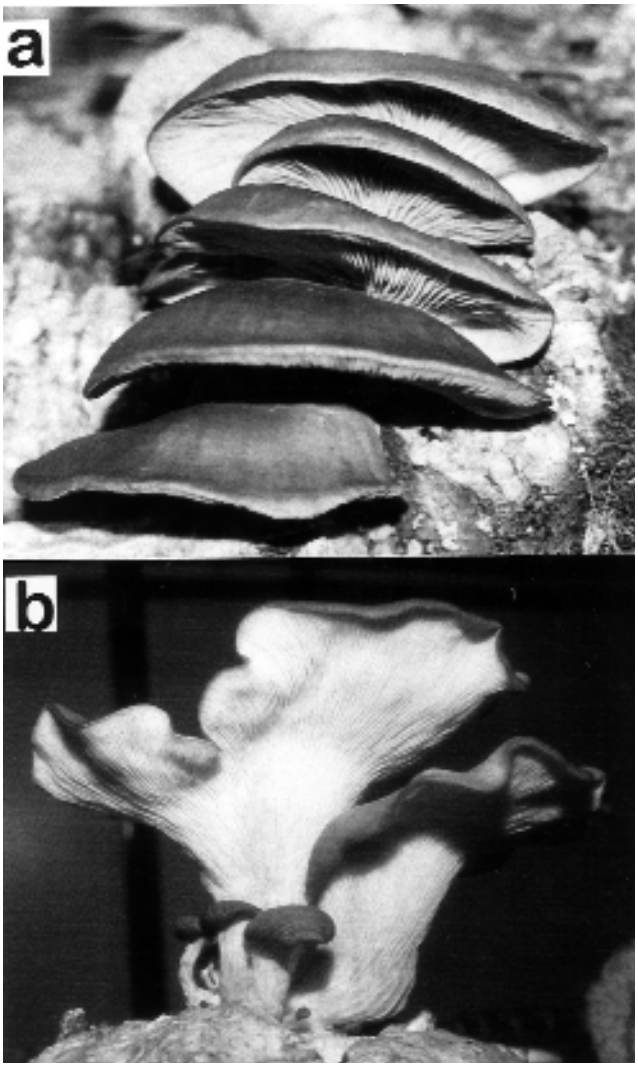

Figure 1. *Pleurotus* from Patagonia. a) Specimens growing on dead trunks of *A. araucana*, b) specimen obtained in culture.

Basidiospores 8-13 x 3.1-3.6 mm, Q: 2.4, n = 20, cylindric, hyaline, thin-walled, inamyloid, smooth (Figure 2). Basidia 29-31 x 7-10 mm, four-spored, narrowly clavate. Cheilocystidia not observed. Pleurocystidia not observed or similar to basidioles. Hyphal pegs absent. Subhymenium 20-25 mm wide. Hymenophoral trama not completely irregular, formed by generative hyphae 2.5-4 mm in diameter and few sclerified 4.5-10.5 mm diameter hyphae (only observed in some specimens). Oleiferous hyphae not observed. Hyphal system monomitic. Hyphae of context, branched, 2.5-6 mm in diameter. Hyphae of stipe thin walled, 3-5 mm, poorly branched, some sclerified, 5-7 mm in diameter. Pileipellis 40-90 mm thick. Hyphae of pileus thin- to thick-walled, pigmented, clamped. Clamp connections present. Exsiccata: pileus tough, smooth, yellowish brown (Munsell 10YR 6/6 to 5/4).

Self-cross. Pairings of 11 monosporic culture BAFC 120 (PPAR) in all combinations revealed a tetrapolar mating system (Figure 3). Mating types were assigned as follows: A_1B_1: isolate 5; A_1B_2: isolates 2 and 9; $A2B_1$: isolates 1, 3, 7 and 11; A_2B_2: isolates 4, 8 and 10.

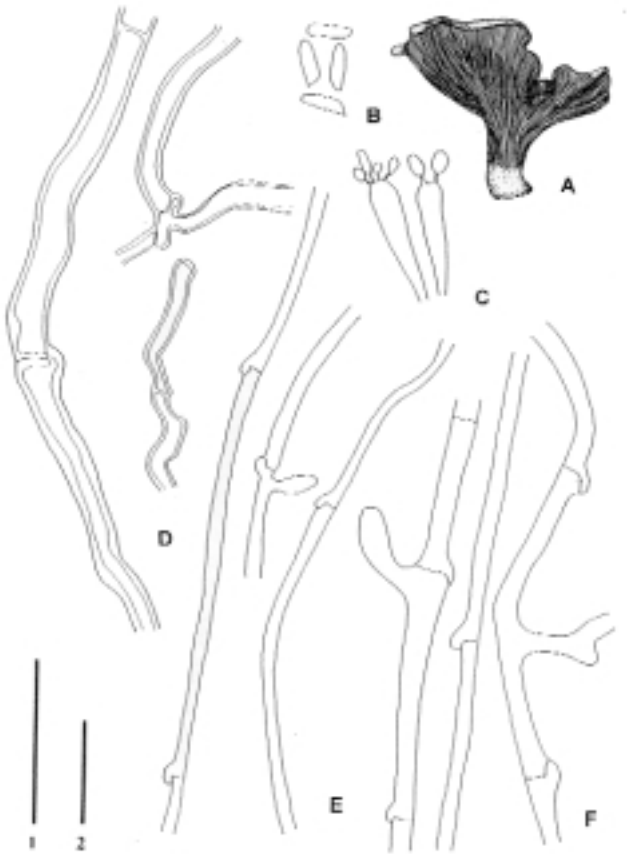

Figure 2. *Pleurotus* from Patagonia. Macro and Micromorphology. A) Basidiocarp; B) Spores; C) Basidia; D) Hyphae of the trama; E) Hyphae of the stem; F) Hyphae of the pileus. Scale Bar 1= 10 cm for A; Scale Bar 2= 20 µm for B-F.

	5	4	8	10	2	9	1	3	7	11
5										
4	+									
8	+	-								
10	+	-	-							
2	-	-	-	-						
9	-	-	-	-	-					
1	-	-	-	-	+	+				
3	-	-	-	-	+	+	-			
7	-	-	-	-	+	+	-	-		
11	-	-	-	-	+	+	-	-	-	

Figure 3. Self-cross grid for *Pleurotus* from Patagonia: +, clump connection present; -, clump connection absent.

OSTR (3505)

PPAR (120)	4	36	15	6
5	+			
2		+		
7			+	
4				+

PULM (4203)

PPAR (120)	1	14	10	9
5	-			
2		-		
7			-	
4				-

Figure 4. Intercollection pairing tests. PPAR: *Pleurotus* sp. from Patagonia; OSTR: Test strain of *P. ostreatus*; PULM: Test strain of *P. pulmonarius*. + paring compatible; - paring incompatible.

Intercollection paring test. Pairings of monosporic strains 5 (A_1B_1), 2 (A_1B_2), 7 (A_2B_1) and 4 (A_2B_2) from *Pleurotus* of Patagonia (PPAR) X 4 mating types of test strains of *Pleurotus ostreatus* (OSTR) and *Pleurotus pulmonarius* (PULM) were carried out. The results of the pairings showed that PPAR is compatible with OSTR and it is not compatible with PULM (Figure 4).

DISCUSSION

Based exclusively on morphological studies, the Patagonian species was determined as *P. pulmonarius*. The general aspect and color of pileus were very similar to the plate published by Bresadola for this species (Iconographia Mycologica, Tab. 293. 1927). Pileus surface was smooth as pointed out by Bas *et al.* [21] for *P. pulmonarius* whereas *P. ostreatus* was shown to present a pubescent-tomentose pileus surface. As regards to microscopic features we found that the size of the pilipellis layer varied from 40-90 µm. According to Hilber [22] *P. pulmonarius* frequently has a pilipellis 40-50 µm thick and *P. ostreatus* a wider one 90-120 µm thick.

In spite of these differences, the interspecific crossing tests showed full compatibility of the Patagonian specimens with *P. ostreatus*. As a consequence, the species from Patagonia is taxonomically determined as *Pleurotus ostreatus* (Jacq.: Fr.) Kumm. The dikaryons isolated were inoculated on sawdust and new sporulating fruitbodies were obtained. This is an important proof to confirm the absolute compatibility between the tester strains and the Patagonian specimens.

Most collectors of specimens of *Pleurotus* commonly use the specific epithet *ostreatus* in Argentina, although we were unable to find published records of this species. Singer & Digilio [2], recorded many polymorphic forms of the "*ostreatus* complex", with transition from the subtropical and tropical forms. These are smaller than the dark dull gray coloured specimens from the warmer areas of Europe. However, they did not point out collecting sites, nor is any record of studied material deposited in local herbaria as *P. ostreatus*. Ramarodi [10] reported a list of species from the province of Buenos Aires, where *P. ostreatus* was included; but he considered the determination as doubtful because the specimens were similar to *P. cornucopiae* Paulet: Fr. In any case no voucher specimens are deposited in any herbaria. As a consequence, the collections studied from Patagonia are the first authentic record of *P. ostreatus* for Argentina.

It is interesting to note that neither Singer [4] nor Horak [11] found this species during their intensive collecting in Patagonia. This may be due to the poor mycological attraction of *Araucaria araucana* as a substrate. Our trips include two visits to pure forests of this species and a mixed forest with *Nothofagus pumilio* (Poepp. & Endl.) Krasser and *N. antarctica* (G. Forster) Oersted. Only *A. araucana* was found to host *P. ostreatus*.

Singer [23] described *P. araucaricola* growing on dead trunks of *Araucaria angustifolia* (Bertol.) Kuntze *(= Araucaria brasiliana* A. Rich). Singer pointed out that "this species, which is closest to *P. ostreatus*, differed from it by the colour of the lamellae and several smaller characters".

Araucaria angustifolia is a species with a subtropical distribution. Although it never grows in cold areas as *A. araucana* does [24] we studied the type specimen of *P. araucaricola* (Brazil, RS, Serra Itaimbezinho 29°, 50,5°. Leg. R. Singer, B 89; 05/XI/51). On the basis of the macro- and microscopic studies we concluded that it could be a synonym of *P. ostreatus* or *P. pulmonarius*.

In fact, it is very difficult, if not impossible, to separate *P. ostreatus* from *P. pulmonarius* based exclusively on micro- and macroscopic features. This indicates the necessity of undertaking other complementary studies such as mating tests or molecular biology to confirm the identity of the species.

This research was financed by the Agencia de Promoción Científica of Argentina (PICT 606) and a Hesler Grant from the Department of Botany, University of Tennessee (to the last author). Publication n ° 141 of the PRHIDEB-CONICET.

References

1. Spegazzini C. Fungi Argentini Novei Critici. An Mus Nac Buenos Aires 1898; 49: 108.
2. Singer R, Digilio PL. Prodromo de la flora agaricina argentina. Lilloa 1951; 25: 6-461.
3. Singer R. Dos especies interesantes de Agaricales en Punta Lara. Bol Soc Arg Bot 1960; 8: 216-218.
4. Singer R. Mycoflora Australis. Nova Hedwigia 1969; 29: 1-405.
5. Raithelhuber J. Hongos Argentinos I. Buenos Aires, Ed. Buenos Aires, 1974.
6. Raithelhuber J. Hongos Argentinos II. Buenos Aires, Ed. Buenos Aires, 1977.
7. Raithelhuber J. Flora Mycológica Argentina; Hongos I. Stuttgart, Mycosur, 1987.
8. Raithelhuber J. Flora Mycológica Argentina; Hongos III. Stuttgart, Mycosur, 1991.
9. Spinedi HA. Primer registro de *Pleurotus smithii* en Argentina. Micol Neotrop Apl 1995; 8: 21-26.
10. Ramarodi E. Flora Micologica de Bahía Blanca y zona (2. parte). Metrodiana 1985; 11: 51-52.
11. Horak E. Fungi Basidiomycetes. Agaricales y Gasteromycetes Secotioides. In: Guarrera SA, Gamundí de Amos I & Rabinovich de Halperin D (Eds.) Flora Criptogámica de Tierra del Fuego. Buenos Aires, CONICET FECIC 1979: 1-528.
12. Mujica FR, Vergara CC, Oehrens EB. Flora Fungosa Chilena. 2nd Ed. Univ. Chile, Fac. Agronomía, Ciencias Agrícolas 1980; 5: 1-308.
13. Farr DF, Bills GF, Chamuris GP, Rossman AY. Fungi on plants and plant products in the United States. Minnesota, American Phytopatological Society Press, 1989.
14. Munsell Color Co Inc. Determination of soil color. Baltimore, US Dept Agriculture Handbook 1954.
15. Kirk PM, Ansell AE. Authors of fungal names. Index of Fungi 1992; Suppl: 1-95.
16. Holmgren PK, Holmgren NH, Barnett LC. Index Herbariorum. New York, New York Botanical Garden, 1990.
17. Nobles MK. Studies in forest pathology VI. Identification of cultures of wood-rotting fungi. Can J Res 1948; 26: 281-431.
18. Burnett JH. Fundamentals of mycology. London, Edward Arnold Ltd. 1968.
19. Zadrazil F. The ecology and industrial production of *Pleurotus ostreatus*, *Pleurotus florida*, *Pleurotus cornucopiae* and *Pleurotus eryngii*. Mushroom Science 1974; 9: 621-652.
20. Stamets PS. Growing Gourmet and medicinal mushrooms. Berkeley, Ten Speed Press, 1993.
21. Bas C. Pleurotaceae. In: Bas C, Kuyper THW, Noordeloos ME, Vellinga EC (Eds.) Flora Agaricina Neerlandica. Rotterdam, AA Balkema 1990; 2: 19-24.
22. Hilbert O. Die Gattung *Pleurotus*. Biblioth Mycol 1982; 87: 1-448.
23. Singer R. Type studies on Basidiomycetes VI. Lilloa 1953; 26: 57-109.
24. Morrone O, Zuloaga F. Gymnospermae. In: Zuloaga F, Morrone O (Eds.) Catálogo de las plantas vasculares de la República Argentina. I. Pteridophyta, Gymnospermae y Angiospermae (Monocotyledoneae). Missouri, Missouri Botanical Garden, 1996: 80-83.