

THE GENUS *GRIFOLA* (APHYLLOPHORALES, BASIDIOMYCOTA) IN ARGENTINA REVISITED

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Summary: *Grifola gargal*, an edible, wood-rotting polypore previously known only from the holotype from Chile, is newly described for southern Argentina. It is restricted to *Nothofagus obliqua*, and it fructifies on wounds of stem and branches of living trees, and on stumps and fallen logs. The morphologies of the basidiomes and cultures are characterized and compared with those of *G. sordulenta*, a species restricted to *N. dombeyi* which fructifies on the soil, and on the butts and stumps of this species. *G. gargal* differs from *G. sordulenta* in having larger spores, and in lacking a central stem; its cultures are slower in growth and form chlamydospores but not fiber hyphae.

Key words: edible fungi, *Nothofagus*, Patagonia, Polyporaceae, taxonomy.

Resumen: El género *Grifola* (Aphyllorales, Basidiomycota) en la Argentina. Se describe para el sur de la Argentina a *Grifola gargal*, un políporo comestible y agente pudridor de la madera que era conocido previamente sólo del holotipo proveniente de Chile. La especie fructifica exclusivamente sobre heridas de fustes y ramas de *Nothofagus obliqua* vivas, y sobre tocones y leño caído. En este trabajo se caracterizan las morfologías de los basidiomas y de los cultivos, y se las compara con aquellas de *G. sordulenta*, especie restringida a los bosques de *N. dombeyi* que fructifica sobre el suelo, y sobre el bajo fuste y tocones de esta especie. *G. gargal* difiere de *G. sordulenta* por sus basidiosporas más grandes, por carecer de un pie central, porque sus cultivos son de crecimiento más lento, y por formar clamidosporas pero no hifas fibrosas.

Palabras clave: hongos comestibles, *Nothofagus*, Patagonia, Polyporaceae, taxonomía.

INTRODUCTION

Grifola S. F. Gray comprises polypores with compound, multipileate basidiomes with numerous, flabellate pilei arising from a common, central base, and fruiting on the soil at the base of trees or stumps. As accepted nowadays (Donk, 1964; Domanski *et al.*, 1973; Gilbertson & Ryvarden, 1986; Ryvarden, 1991, among others) the genus contains monomitic species with clamped generative hyphae (but cfr. Ryvarden, 1991, who described the hyphal system as dimitic), ovoid to ellipsoid, smooth, inamyloid spores, and producing a white rot in the substrate. This has restricted the original broad concept of the genus (Gray, 1821; Pilát, 1936) to only a few species, in spite of wider interpretations by some authors (e.g., Cunningham, 1965).

So far, *Grifola* has a rather isolated position in the system of polypores. Jülich (1981) created Grifolaceae, with *Grifola* and *Abortiporus* Murrill, and included it in the order Grifolales together with the family Meripilaceae (Jülich, 1981, with *Meripilus*

P. Karst. as its only genus). Singer (1969) included the genus in Scutigigeraceae Bondartsev & Singer but this family has a narrower definition (Hawksworth *et al.*, 1995). Corner (1989) considered that *Grifola* was related to the genus *Albatrellus* S.F. Gray, a terricolous and mycorrhizal taxon, and neglected any relationship with *Meripilus* on account of the sarcodimitic construction in the latter. Hawksworth *et al.* (1995) placed the genus in the Coriolaceae. Phylogenetic studies based on molecular characters of polypores and other aphylloraceous fungi have only recently included species of this genus (Hibbett *et al.*, 2000; Hibbett & Donoghue, 2001). They have shown that the type species, *G. frondosa* (Dicks.: Fr.) S.F. Gray, either belongs to a white wood-rotting clade named as the 'Phlebia clade' or to a brown wood-rotting clade named as the 'Antrodia clade'. Thus, it is still difficult to ascertain the possible relationships of this genus with other taxa in the system until more studies are undertaken.

Most of the species in the genus have been described from temperate areas in the southern and northern hemispheres, but Shaw (1984), Corner (1989), and Quanten (1997) have described several species from the tropics in Papua New Guinea, Singapore

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and Borneo; and Hattori (2000) transferred one of Corner's tropical species to the genus. For all of South America, the genus has only been reported from the *Nothofagus*-dominated forests of southern Argentina and Chile. Singer (1969) and Wright & Deschamps (1972) recorded and/or described *G. frondosa*, *G. gargal* Singer and *G. sordulenta* (Mont.) Singer from that area. The study of species in this genus may have a technological application, as the type species has been claimed to have several medicinal properties (Wasser & Weiss, 1999). *G. frondosa* is a significant commercial edible mushroom especially in Japan, where it is widely available in supermarkets as a fresh product as well as dried. The aim of this study is to give full accounts of the species so far found in Argentina in order to improve knowledge of their characteristics, distribution and host characteristics.

MATERIAL AND METHODS

Freehand sections of dried herbarium material were mounted in 3-5% KOH plus aqueous phloxine, in lactophenol, and in Melzer's reagent (reaction amyloid, dextrinoid or IKI-). The material was also examined in a 1% aqueous solution of cresyl blue to test for a metachromatic reaction (purple coloration) in hyphae. Cultures were obtained from context tissue of fresh fruit-bodies and/or from germinating spores obtained from a spore print. Culture features were studied, described and coded according to the system of Nobles (1965) and Nakasone (1990). Line illustrations of microscopic features were made using a drawing tube. Herbaria abbreviations follow Holmgren *et al.* (1990).

RESULTS

Grifola gargal Singer, Mycoflora Australis p. 381, 1969.
Figs. 1A, 2

Holotype: CHILE. Valdivia, 15 km NE of El Mirador, Cordillera Pelada, 6.May.67, leg. Miranda comm. R. Singer M6934 (SGO 092562!).

Basidiocarp annual, multipileate, formed by numerous pilei rosette-like or cabbage-like, imbricated, growing from a common point, some pilei developing from the base or from the laterals of other pilei; odor

strongly of anised almond when fresh, fading upon drying; tasty and fleshy, up to 30 cm diam. Pilei sessile, flabellate, with or without an attenuated base but never developing a true stipe, applanate to convex, small to large, up to 8 cm wide x 7 cm radius x 1.5 cm thick; margin thinning out and sometimes incurving, straight to lobate. Pilear surface glabrous, fibrillar, cream yellowish, light beige, light chestnut or greyish; sometimes with pubescent areas, rarely also with scrupose areas composed of tufts of coalescing, regularly arranged hyphae. Hymenophore initially poroid, with the pores angular and radially elongated, afterwards becoming foliose-lamellate and split, and also markedly dentate or irregularly poroid, with the pore mouths distinctly split to irpicoid, creamish white when fresh turning straw colored upon drying, 1-2/mm. Context creamish white, up to 10 mm thick. Tubes white, up to 5 mm long.

Hyphal system monomitic. Generative hyphae clamped, 3-5 μm diam. in the dissepiments, with thin to slightly thickened, hyaline, metachromatic walls, generally empty, few with cytoplasm; hyphae in the context 4-7-10 μm diam., branched, regular or swollen up to 15-20 μm diam., especially so near or around the clamps, with slightly thickened or up to 1-2 μm thickened, hyaline, metachromatic walls, abundantly septate and recalling a puzzle, most of them devoid of cytoplasm.

Basidia claviform, sometimes robust, 25-35 x 7-8 μm , with 4 sterigmata up to 6 μm long.

Basidioles cylindric to claviform, 20-35 x 6-8 μm .

Cystidia absent.

Basidiospores ellipsoid to broadly ellipsoid, with a straight inner side that is not always evident, 7-8 x 5-6 μm , with smooth, hyaline, thin to slightly thickened, IKI-, acyanophilous walls, with a large guttule or many small ones in the cytoplasm, abundant.

Associated wood-rot a white pocket rot with a strong almond scent.

Substrate: on living trunks and large branches fruiting on wounds, and on stumps or on fallen trunks of *Nothofagus obliqua*.

Pathology: possibly an important heartwood decayer of standing *N. obliqua*.

Distribution: endemic species in the *N. obliqua* dominated forests of southern Argentina and south-central Chile. Originally described from south of Valdivia in Chile (Singer, 1969).

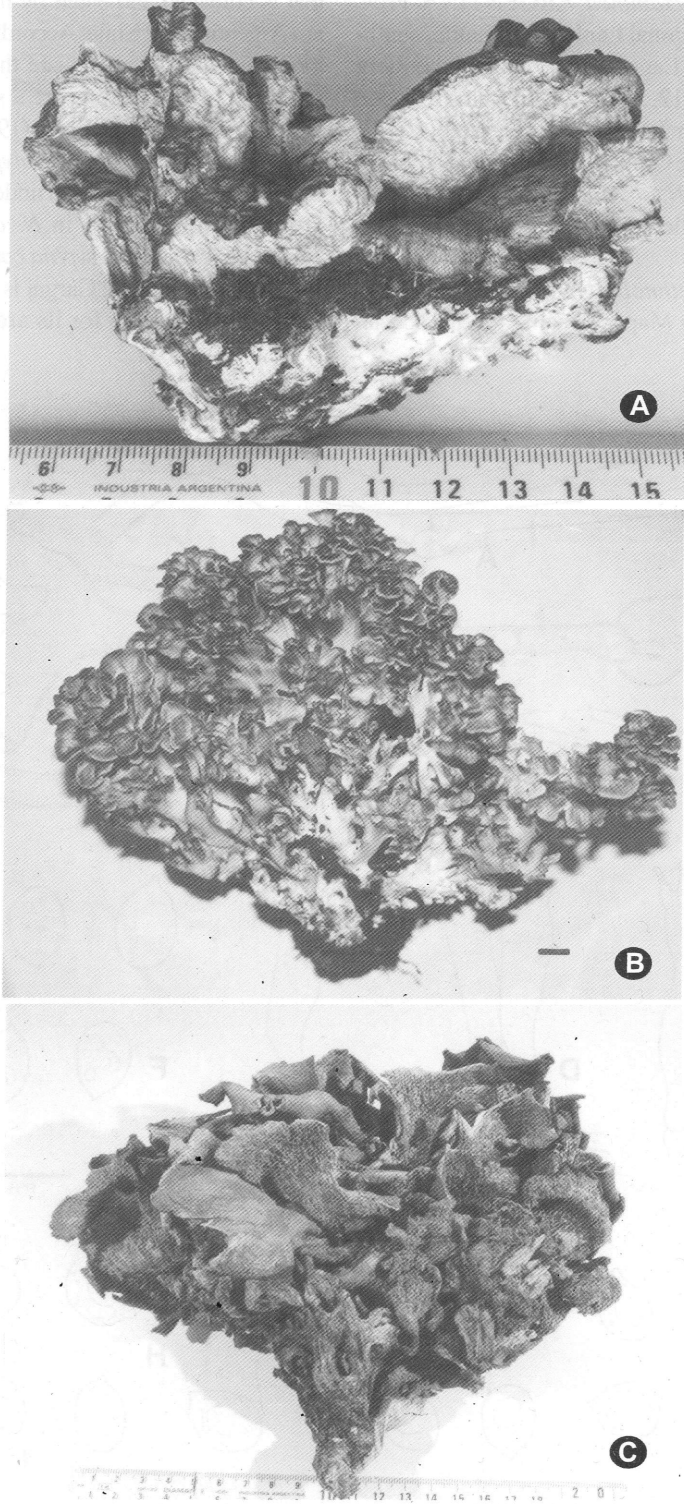


Fig. 1. Basidiomes of *Grifola* species from southern Argentina. A: *Grifola gargal* (specimen MR 11107). B-C: *Grifola sordulenta*, B: fresh and young fruiting body (specimen MR 11990); C: dried and mature fruiting body (BAFC 25572). Bar and rules in cm.

Other collections examined: ARGENTINA. Prov. Neuquén: Parque Nacional Lanín, Lago Lanín, southern margin, road to Lago Queñi, M. Rajchenberg 11107, 11109 and 11112, 28-IV-96. *Ibid.*, in *N. obliqua* forest ca. 1 km from Lago Queñi, H. Mattes (MR 11911), 19-V-99. Lake Quillén, ca. the campsite, 11-V-2000, on a wound of *N. obliqua* stem at 5 m high, M. Rajchenberg (not collected).

Etymology and ethnobotany: “gargal” (variant “galgal”) is the native Mapuche name for this species

that is collected and eaten by native people of southern Argentina and Chile. According to Baeza (1930) the mountain Indians appreciate this winter-fruiting fungus a lot, using it to prepare a stew or roasting it in a barbecue with salt. Both Baeza (1930) and Gunckel (1959) established that it was a *Polyporus* sp., but Smith-Ramírez (1995) mentioned under this name a *Ramaria* sp. that fruits on soil in *N. obliqua*, *Weinmannia trichosperma* and *Eucryphia cordifolia* forests; it must be a different fungus. Gargal is a tasty and fleshy fungus most appealing for its aromatic scent.

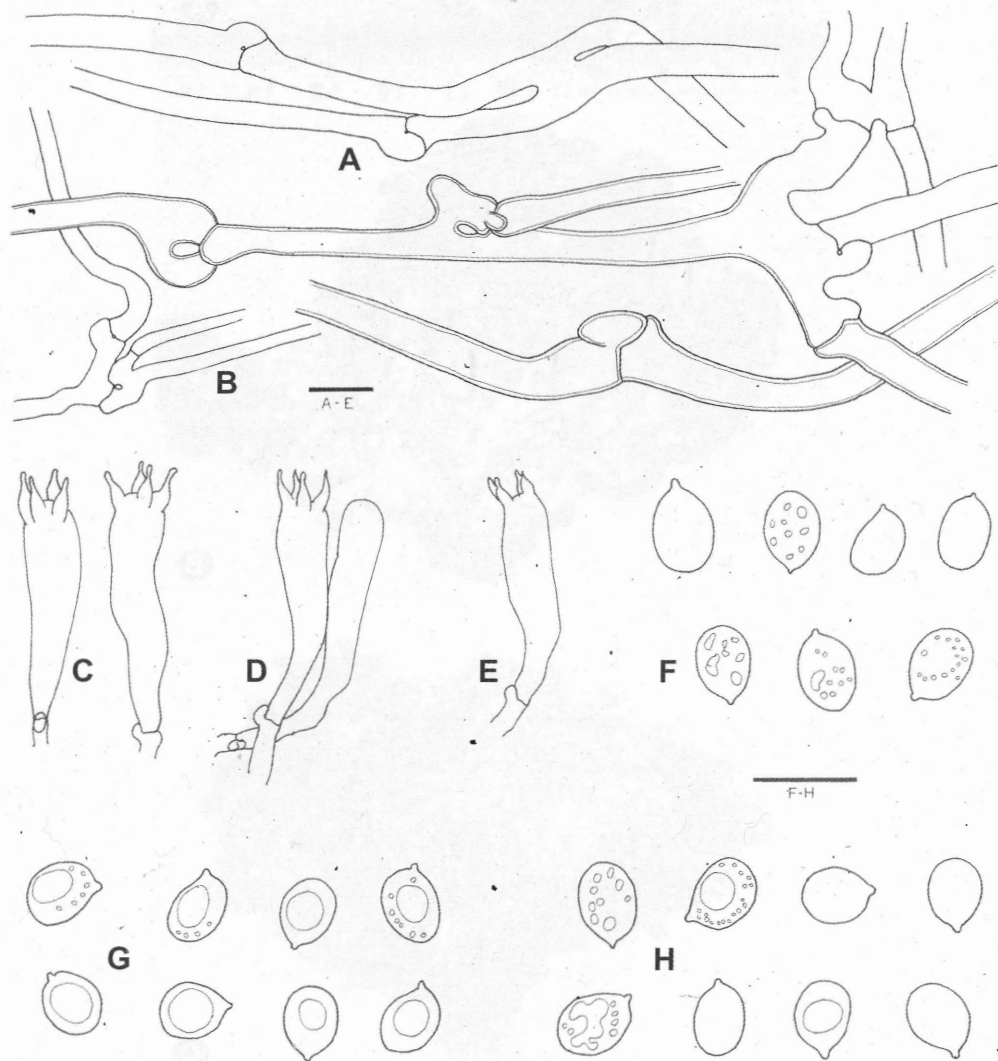


Fig. 2. Microscopic characters of *Grifola gargal* basidiomes. A-B: generative hyphae from the context (A) and from the dissepiments (B). C-E: Basidia from specimens MR 11107 (C), MR 11911 (D) and holotype at SGO (E). F-H: Basidiospores from holotype at SGO (F), and specimens MR 11911 (G) and MR 11107 (H). Bars= 10 μm

Remarks: This is the second report of the taxon since its original description. Wright & Deschamps (1972) did not find it during their survey of polypores from Patagonia but the fungus seems to fruit regularly in the area and, Rajchenberg (2001) recorded it recently. The type in SGO is in poor condition, being small, very thin and papyraceous broken, and with only a few, small sectors that bear a hymenophore. Nevertheless typical basidiospores are present.

Cultural characters (Figs. 3A, 4)

Cultures studied: CIEFAP 190 (=MR 11112), CIEFAP 191 (=MR 11109) and MR 11911.

Macroscopic features: Growth very slow, hardly reaching 3 cm in radius at the end of 6 weeks; mat felty-farinoso in the aerial marginal area, translucent, becoming dense and white towards the inoculum; marginal mycelium also growing into the agar; margin regular. Reverse bleaching. Odor strongly almond.

Oxidases reaction: gallic acid +, 1 cm diam., tannic acid ++, tr.; tyrosinase -, 2 cm diam.

Microscopic characters: Marginal hyphae clamped, 2.5-5 μm diam., with thin to slightly thickened walls, back from margin becoming wider, 4 - 7 μm diam. Chlamydospores terminal or intercalary, globose to subglobose, some pip-shaped or almond-shaped, with or without a lateral foot, 8 - 16 x 8 - 12 μm , with slightly thickened, hyaline walls. Allocysts apparently present, but probably representing immature states of chlamydospores. White mycelial mat around the inoculum

formed of intertwined hyphal segments of generative hyphae that form a net; these are mostly devoid of cytoplasm, encrusted, with clamps that are difficult to distinguish, and 4-8 μm diam.

Species Code: 2a.3.7.(26).34.36.40.47.53.54.58.

Sexuality: heterothallic, but type of incompatibility system not known. Only three monosporic cultures were obtained (from specimen MR 11911) and gave the following result: 1 x 2 = -, 1 x 3 = +, 2 x 3 = -.

Grifola sordulenta (Mont.) Singer, The Agaricales in Modern Taxonomy p. 300, 1962.

Figs. 1B - C, 5

=*Polyporus sordulentus* Mont. in Gay, Hist. Fis. Pol. Chile, Bot. 7: 357, 1850.

Holotype: CHILE (PC!).

Basidiocarp multipileate, formed of more than 40 pilei that are arranged rosette-like or cabbage-like, forming a structure of up to 35 cm wide x 15 cm in radius x 30 cm in height, the pilei forming one after the other from a common or unique base or trunk, more or less well developed, but branching soon after their formation, each branch forming a different pileus that may or may not fuse laterally with another one, the pilei imbricately arranged. Pilei flabellate, convex, with an attenuated base that fuses with those of other pilei, small or up to 8 cm wide x 8 cm in radius x 0.7 cm thick, but most of them more or less uniform in size.

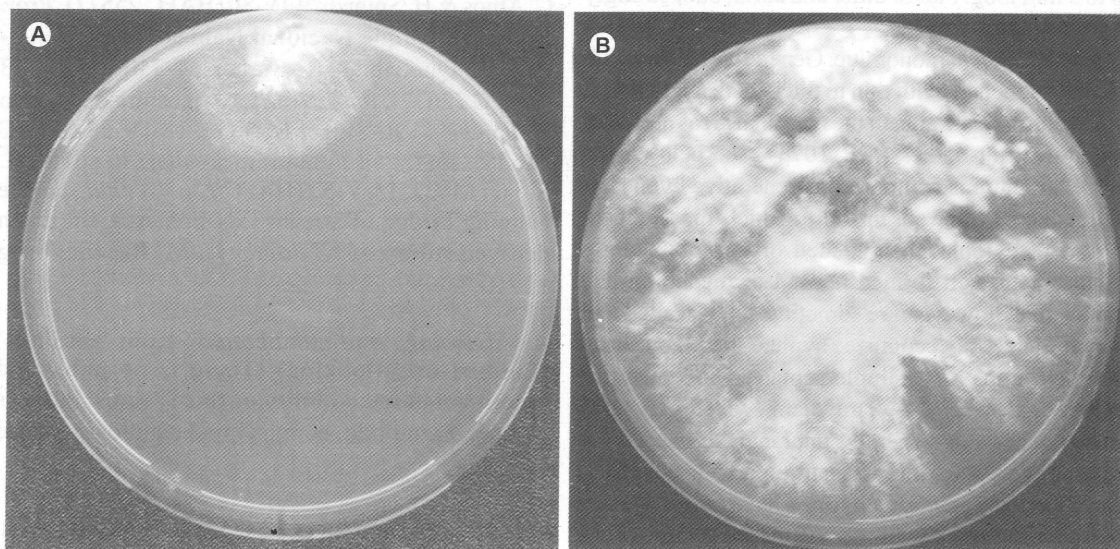


Fig. 3. Macroscopic aspect of *Grifola* species in culture at week 6. **A:** *Grifola gargal* (culture CIEFAP 191). **B:** *Grifola sordulenta* (culture CIEFAP 154; cfr. Rajchenberg & Greslebin, 1995). Dishes are 9 cm diam.

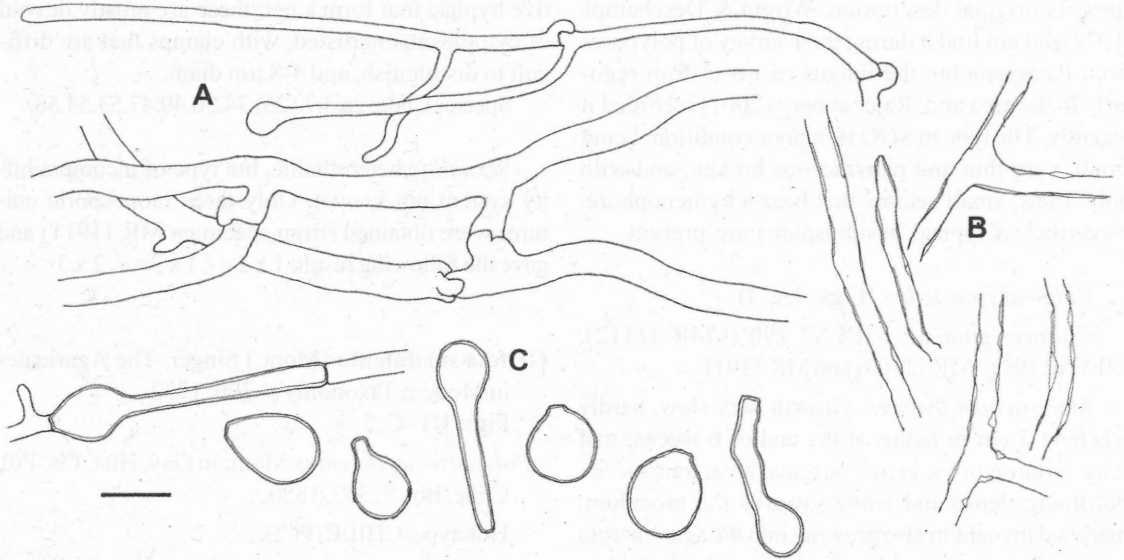


Fig. 4: Microscopic characters of *Grifola gargal* cultures. A: generative hyphae. B: encrusted hyphae from the inoculum area. C: chlamydospores. Bar= 10 μ m.

Pilear surface glabrous, smooth, with or without delicately pubescent areas or with agglutinated tufts of hyphae, or with radially arranged fibrils or veins, cream, light cinnamon or greyish. Margin regular, thinning out, rarely with festoons. Hymenial surface creamish white, poroid, with the pores angular, isodiametric or elongated, or poroid-lacerate to lamellar, with entire or serrulated borders, 1-2 /mm. Context creamish, fleshy, tenacious, up to 3 mm thick. Tubes up to 5 mm long. Flavor bitter and acrid, odor strongly of anise (Singer 1969).

Hyphal system monomitic. Generative hyphae with clamps, branched, with thin to thickened, hyaline, IKI -, metachromatic walls. In the marginal areas and/or in immature fruit-bodies (MR 10737, see below), the whole structure is composed of generative hyphae 4-10 μ m diam., with walls up to 1 μ m thick, most of them devoid of cytoplasm, branched or not, abundantly septate, with well formed normal or medallion-like clamps that, however, are sometimes thin-walled and hard to detect or recognize; hyphae then, appearing to lack clamps as if simple septate. Gloeopleurous hyphae also present, 4-10 μ m diam., with thin or up to 1 μ m thick walls, generally unbranched, intensively staining with phloxine. In mature parts of the pileus and stipe the generative hyphae have walls 2-3 μ m thick, with the inner border of the wall uniform or irregular; in KOH walls appears southern Argentina and Chile. It fruits rather rare.

Other collections examined: ARGENTINA. *Prov. Chubut:* Parque Nacional Los Alerces, Lake Menéndez, milenary *Fitzroya cupressoides* stand, on the base of a standing *N. dombeyi*, M. Rajchenberg 10737, 15-III-93. *Prov. Río Negro,* Parque Nacional Nahuel Huapi, Puerto Blest, on soil in *Nothofagus dombeyi* forest, leg. R. Singer M-3145, 23-III-63 (BAFC 22714). *Ibid.*, G. Mueller, 25-III-87 (BAFC 30872). *Ibid.*, on roots of *N. dombeyi*, C. Amos & H. Spinedi, 9-IV-80 (BAFC 25572). *Prov. Neuquén:* Parque Nacional Nahuel Huapi, Península de Quetrihué; R. Singer M-3300, 7-IV-63 (BAFC 22637). *Ibid.*, Villa La Angostura, 'ad basim *N. dombeyi*', R. Singer M-5032, 18-IV-65 (BAFC 22628). *Ibid.*, 'ad lignum *N. dombeyi* in silva', R. Singer M622, 15-V-52 (BAFC 22630). *Ibid.*, ca. the hanging bridge of stream Los Cántaros, on much decayed stump of *N. dombeyi*, M. Rajchenberg 11910, 8-V-99.

Etymology and ethnobotany: from 'sordidus' (Latin = dirty-looking). There is no published record about the edibility of this species, but it is tasty and aromatic, being a good candidate for the table.

Cultural characters: Rajchenberg & Greslebin (1995) (cf. Fig. 3B for macroscopic aspect).

Species code: (1).2.3.8.32.36.40.47.53.54.

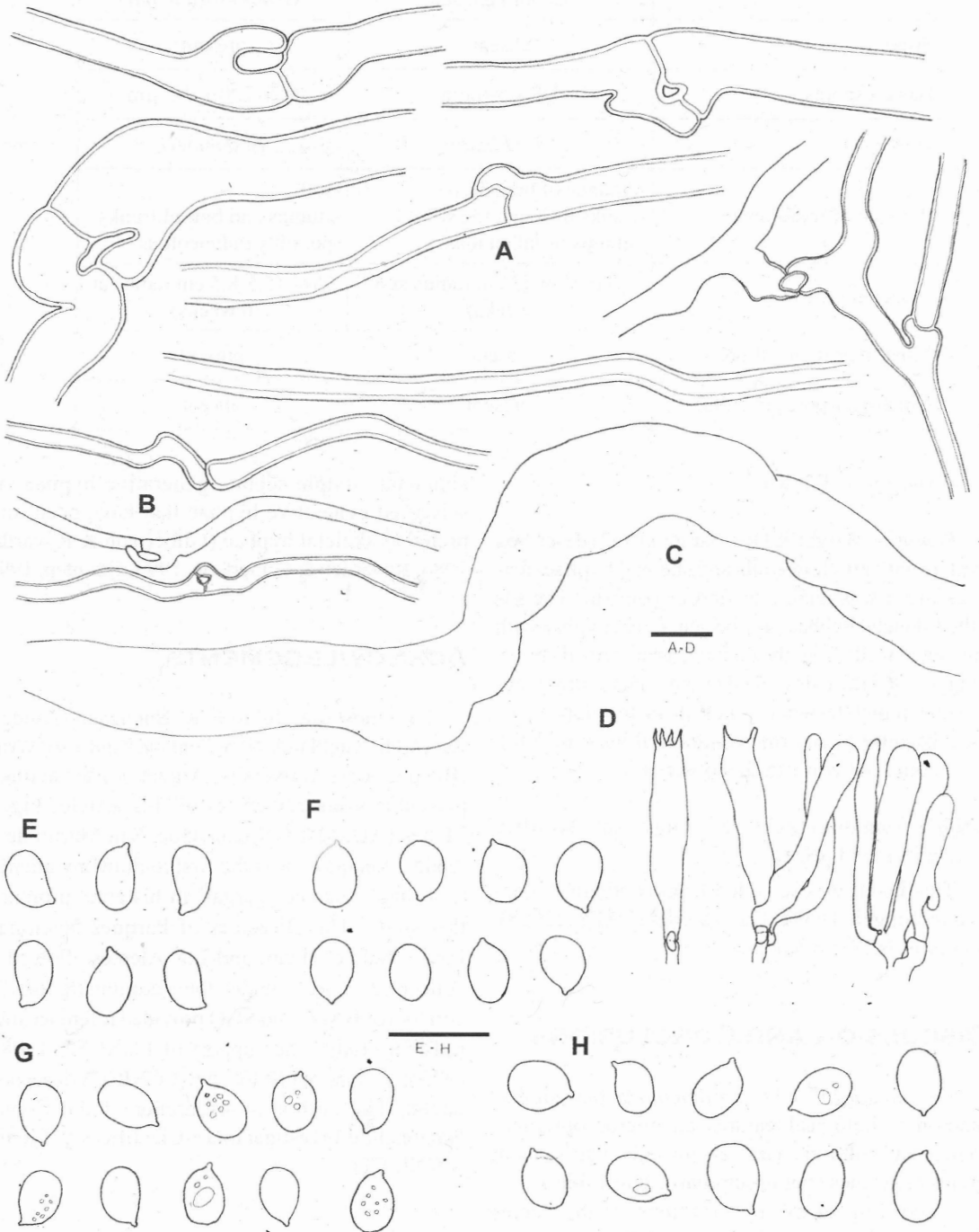


Fig. 5. Microscopic characters of *Grifola sordulenta* basidiomes. **A-B:** thin- and thick-walled generative hyphae from the context (A) and from the dissepiments (B). **C:** gloeopleurous hyphae from the context. **D:** basidia (specimen BAFC 22630). **E-H:** basidiospores from holotype at P (E), and specimens BAFC 22628 (F), BAFC 22714 (G) and BAFC 22630 (H).

Table 1. Features that distinguish *Grifola* species in southern Argentina.

	<i>Grifola gargal</i>	<i>Grifola sordulenta</i>
Stipe	absent	present
Basidiospores	7-8 x 5-6 µm	6-7(-7.5) x 4-5 µm
Host	<i>N. obliqua</i>	<i>N. dombeyi</i>
Position of basidiomes	-wounds of branches -trunks of living trees -stumps or fallen logs	-soil -stumps and butt of trunks -possibly radicicolous
Mycelial growth	very slow (3 cm radius at 6 weeks)	slow (5.5-8.5 cm radius at 6 weeks)
Fiber hyphae in culture	absent	present
Chlamydospores in culture	present	absent

Sexuality: unknown.

Remarks: Wright & Deschamps (1972) described hyphae with amyloid walls and skeletal hyphae, features that I was unable to find or confirm. The so-called skeletal hyphae may be generative hyphae with thickened walls from the context, as described above. Singer (1969) also described ornamented spores ("appearing punctate when focused on the upper surface, because of an ornamentation of the type XI"), but I could not confirm this observation.

Grifola frondosa (Dicks.: Fr.) Gray, Nat. Arr. Brit. Plants 1: 643, 1821.

This taxon was recorded from southern Argentina by Singer (1969). The material (BAFC 22628), however, is *G. sordulenta*.

DISCUSSION AND CONCLUSIONS

Grifola gargal and *G. sordulenta* display similar macromorphological features, but microscopic, ecological and cultural characters may distinguish them quite easily, these being summarized in Table 1.

According to my observations, both species have a monomitic hyphal system. This is also the case in the type species, the north temperate *G. frondosa*, and other species described from the tropics by Corner (1989). *Grifola frondosa* differs in having smaller pores, 2-4/mm, secondarily formed,

abundant, simple septate generative hyphae, and sclerified generative hyphae that have been interpreted as skeletal hyphae (Gilbertson & Ryvarden, 1986; Ryvarden & Gilbertson, 1993; Quanten, 1997).

ACKNOWLEDGMENTS

I am most grateful to P. K. Buchanan (Landcare Research, Auckland, New Zealand) and J. E. Wright (Buenos Aires University, Argentina) for acting as pre-submission reviewers of this article. Ing. H. Mattes (AUSMA-UN Comahue, San Martín de los Andes, Neuquén) was the first to draw my attention to a fungus named 'gargal' in his area, prompting this study. The Directors of Parques Nacionales Lanín, Nahuel Huapi and Los Alerces allowed the collecting in areas under their administration. The curators of BAFC and SGO provided reference materials for study. The support of FONCYT 1908/97 (ANPCYT) and of PIP 4062/96 (CONICET) is acknowledged. The author is researcher of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET).

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Recibido el 15 de Octubre de 2001, aceptado el 02 de Febrero de 2002.