Gautieria inapire sp. nov., a new hypogeous species from Nothofagus forest in southern Central Chile

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The macroscopic and microscopic features of the new species *Gautieria inapire* (Basidiomycota), discovered in an Andean *Nothofagus* forest in southern Central Chile, are described and its taxonomic position and relationships within the genus and related taxa are discussed. This species with hypogeous basidiomata is characterized by its capacity to form ectomycorrhiza with *Nothofagus pumilio* (Poepp. & Endl.) Krasser; by the dark brown peridium \pm persistently enclosing the basidiomata and by the relatively small, longitudinally costate basidiospores, dimpled by an apical depression.

Keywords: Gautieria, Basidiomycota, Nothofagus pumilio, Chile.

The subantarctic *Nothofagus* forests of Chile and Argentina represent the largest natural and coherent ectomycorrhizal area in the Southern Hemisphere (Singer & Morello, 1960). It is characterized by numerous, endemic macrofungi (Singer, 1969; Horak, 1979; Garrido, 1988) which are known to form ectomycorrhizae with the eleven recognized taxa of southern beeches (Vázquez & Rodrìguez, 1999). In the temperate rain forests of southern South America (extending for about 2000 km in S-N direction), *N. pumilio* ("Lenga") shows the widest distribution of all *Nothofagus* species, its area of distribution (Hildebrand-Vogel & al., 1990) extending from 35° (Tierra del Fuego) northwards to 55° south. lat. (Patagonian Andes, Cordillera of Central Chile).

In general, little is known about the diversity and ecology of the epigeous or hypogeous macrofungi occurring in temperate, subantarctic forests in the southern Andes. The new hypogeous species described here, *Gautieria inapire*, is only the second taxon recorded in the area (Dodge & Zeller, 1934; Horak, 1964; Castellano & Trappe, 1990). It was found at timberline in a pure stand of *N. pumilio* on the Chilean side of the Andes (Prov. Osorno).

Taxonomy

Gautieria inapire Palfner & E. Horak, sp. nov. – Pl. 1: 1–2; Figs. 1–4.

Basidiomata 5–30 mm lata, hypogaea, irregulariter globosa vel tuberiformia, peridio brunneo velutinoque persistenter obtecta. Gleba cerebriformis, ex cellulis irregulariter labyrinthicis composita, albidula, mox cinereobrunnea, haud gelatinosa, columella dendroidea. Rhizomorpha externa ad basim ex hyphis partiter inflatis obtecta. Cystidia claviformia vel irregulariter inflata, acanthohyphae praesentes. Odor fortis, aromaticus, haud ingratus. Statismosporae $(12-)14-17(-21) \times (8-)9-12(-14) \mu m$, ovoideae vel ellipsoideae, basim versus attenuatae, (6-)7-9 costis longitudinalibus furcatisque instructae, apicaliter umbilicatae, (sub-)hyalinae, inamyloideae. Basidia $(25-)35-45(-70) \times 4-8 \mu m$, 2–4 sporigera, cylindrica vel clavata, hyalina. Endocystidia nulla. Fibulae nullae.

Subterranea in silvis nothofagineis (*N. pumilio* et *Drimys winteri* var. *andina*), Chile, Prov. Osorno, Parque Nacional Puyehue, Refugio Antillanca, 27 Mar. 1998, leg. Palfner GP 4901 (Holotypus, M).

Basidiomata 5-30 mm diam., hypogeous in litter but close beneath surface, solitary or confluent, irregularly tuberiform, subglobose or broadly kidney-shaped, firm, attached to a distinct, stromatic layer of white mycelium, solitary or in small groups, base covered with occasional, thin rhizomorphs and a single stipe-like mycelial strand (up to 10 mm long and up to 2 mm in diam.) connected with fan-like, thinner rhizomorphs to the substrate, breaking off easily and exuding transparent, watery latex, leaving a dark ring around whitish center, soon staining grevish brown (Fig. 1). -Peridium velvety to tomentose, dark brown to fuscous, at first persistently covering gleba but in ageing specimens becoming discontinuous with glebal locules showing through or exposed. -Gleba composed of folded, labyrinthiform cavities, up to 0.5 mm in diam., chambers embedded in an transparent layer of gelatinouscartilaginous substance, whitish or pale yellowish when cut, gradually becoming greyish-brown with reddish tint. - Columella distinctive, central, dendroid, about 2 mm at base, gradually ramified towards peridum, whitish, tramal plates whitish. - Odor strong, aromatic, with distinctive turpentine component. - Taste not recorded.

Spore mass yellowish brown. – Statismospores (12–) $14-17(-21) \times (8-)9-12(-14) \mu m$ (excluding ridges), symmetrical, ellipsoid with elongate basal apiculus, exospore folded into (6-)7-9 distinctive, bluntly edged, longitudinal, $1-2 \mu m$ high and 2-4 (-5) μm broad ridges, usually meridian-like but occasionally slightly coiled, often bifurcate and confluent with neighbouring ridges, frequently interconnected with thin anastomoses, ridges terminating at flat apex with distinctive, dimple-like depression, walls pale brown to golden brown, inamyloid, basal apiculus often with hyaline, several μm long remnant of the sterigma (Pl. 1, 3–4). – Basidia (25–)35–45(–70) × 4–8 μm , cylindrical or subclaviform, thin-walled,

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Pl. 1. – SEM-photographs of statismospores (x3000, bar = 5 µm). – 1, 2. Gautieria fuegiana E. Horak (holotype). – 3, 4. Gautieria inapire Palfner & E. Horak (holotype).



Pl. 2. – SEM-photographs of statismospores (x3000, bar = 5 μ m)– 1. *Gautieria novaezelandiae* G. Cunn. (ZT 68–674). – 2. *Chamonixia caespitosa* Rolland (ZT 1973).

with 2, 3 or 4 sterigmata, thin-walled, often filled with small granules or droplets, usually collapsed in dry material, difficult to rehydrate in KOH, water or cotton blue-lactic acid, clamp connections absent (Fig. 2: 2). - Endocystidia absent. - Trama subregular, composed of cylindrical hyphae, $100-150 \times (1-)2-3$ (-4) µm, clampless, at septa sometimes slightly inflated, then up to 5 µm in diameter, hyaline, some segments or hyphal ends sinuous, with yellowish brown deuteroplasma, strongly staining in cotton blue (Fig. 2: 1). - Peridium with numerous large cystidia or cystidia-like inflated hyphal ends, polymorphic, hyphae ranging in shape from narrowly cylindrical to broadly rounded or oval, often with an apical protuberance, yellowish brown to dark brown, thickwalled membranes, occasionally intermixed with thin-walled, intrahyphal hyphae (Fig. 3: 2). – Mycelial hyphae close to base of basidiomata hyaline, often with thickened cell walls, occasionally with yellowish-opaque, amorphous incrustations, often agglutinated. - Rhizomorphs at base of basidiomata often with inflated hyphal sections and polymorphic, large cystidia (like those of peridium), rarely with thin acanthohyphidia (Fig. 3: 1, Fig. 4: 1–3).

Etymology. – "*inapire*" (in Mapudungún, language of native Chileans, cf. Mösbach 1992): "Close to the snow", relating to the type locality which is close to the timberline on the volcano Casablanca.



Fig. 1. – Gautieria inapire Palfner & E. Horak (holotype). – 1. Basidiomata (nat. size, bar = 20 mm).

Habit and habitat. – Hypogeous in leaf litter and debris, sometimes exposed, gregarious, originating from white mycelial mats extending into humus and litter above volcanic ash. Found at about 1100 m a.s.l. at timberline in a pure stand of *Nothofagus pumilio* (Poepp. & Endl.) Krasser, associated with *Drimys winteri* var. *andina* Reiche in the undergrowth.

Material examined. – Gautieria inapire Palfner & E. Horak: Chile: X. Región, Provincia de Osorno, Cordillera de los Andes, Parque Nacional Puyehue, Refugio Antillanca (40°42'S, 72°10'W), ca. 1100 m above sea level, annual precipitation between 4500-5000 mm, 27 Mar. 1998, leg. Palfner GP 4901 (holotype M; isotype ZT 7255; paratype ZT 7256). – Gautieria chilensis Zeller & Dodge (1934): Chile: Prov. Magallanes, Punta Arenas, Feb. 1906, leg. Thaxter, det. Dodge, nr. 14 (holotype, FH 4584). – Gautieria fuegiana E. Horak (1964): Chile: Prov. Magallanes, Monte Alto, Los Robles, under N. pumilio, 25 Mar. 1963, leg. Horak (holotype, ZT 64–92). – Gautieria novaezelandiae G. Cunn. (1938): New Zealand: North Island, TO, Chateau, Apr. 1931, leg. Carter (PDD 8331, ZT 68–674).

Discussion

About 30 taxa belonging to *Gautieria* Vitt. (1831) have been described world-wide. Representatives of this secotiaceous genus vare reported from both the northern (Trappe & Guzmán, 1971; Gross & al., 1980; Montecchi & Lazzari, 1993; Pegler & al., 1993; Fan & al., 1994) and southern (Cribb, 1957; Beaton & al., 1985; Stewart & Trappe, 1985, as "Austrogautieria") hemispheres. The most characteristic and conspicuous feature of *Gautieria* species (with rather close taxonomic relationships to Gautieria, J. Trappe

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Fig. 2. – *Gautieria inapire* Palfner & E. Horak (holotype). – 1. Tramal plate (in cross section) composed of cylindrical, with weakly gelatinized hyphae, yellowish pigment occasionally present, hyphal ends often inflated. – 2. Mature and immature basidia with statismospores in different stages of development (bar = $20 \mu m$).



Fig. 3. – Gautieria inapire Palfner & E. Horak (holotype). Characters found at base of basidiomata. – 1. Rhizomorph with partly swollen, thick-walled hyphae and cystidia. – 2. Peridial cystidia of different shape and size (bar = $20 \mu m$).



Fig. 4. – Gautieria inapire Palfner & E. Horak (holotype). Characters of mycelium at base of basidiomes. – 1. Hyphae with irregularly thickened, partly gelatinized walls, with hyaline to yellowish, subglobose appositions. – 2. Rhizomorph with single, yellow pigmented acanthocystidium. – 3. Acanthocystidia of different shape and size (bar = $20 \mu m$).

pers. comm.) are the longitudinally ridged statismospores (Pl. 1: 1-4. Pl. 2, 1), which are clearly seen only in the SEM (Pegler & al., 1993).

Using the light microscope only, the statismospores of the bolete-related *Chamonixia caespitosa* Rolland (1899) can also be readily confused with those of *Gautieria*. In the SEM, however, the microstructures of the broad, longitudinal ridges (Pl. 2: 2) are distinctly different from those observed in species of *Gautieria*.

Based upon ecological evidence it is assumed that all of the strictly hypogeous basidiomata of *Gautieria* produce ectomycorrhiza with (evergreen or deciduous) coniferous and broadleaved trees (Garrido, 1988). The majority of described species have been recorded from Australia where *Gautieria* is predominantly found growing under *Eucalyptus* (Cribb, 1957; Beaton & al., 1985).

So far, only two species occurring in subantarctic, Andino-Patagonian *Nothofagus* forests (both associated with *N. pumilio*) have been reported (Singer, 1969; Horak, 1979; Halling, 1981; Castellano & Trappe, 1990), viz. *G. chilensis* Zeller & Dodge (in Dodge & Zeller, 1934), originally described from Punta Arenas, and *G. fuegiana* Horak (1964), also reported from the Magallanes Province in southern Chile. The re-examination of the type material of *G. fuegiana* (Pl. 1: 1–2) revealed, however, that this taxon is actually a later synonym of *G. chilensis*.

The new taxon G. *inapire* is readily distinguished from the sympatric G. *chilensis* by its significantly smaller statismospores which have more numerous and more conspicuous longitudinal ridges. In addition the statismospores of G. *chilensis* lack the distinctive apical dimple or callus (Pl. 1, 1–4).

Another southern hemisphere species of *Gautieria*, also associated with *Nothofagus* spp., is *G. novaezelandiae* G. Cunn. (1938). The basidiomata of this New Zealand species share several macroscopic and ecological characters with *G. inapire*. However, the statismospores of *G. novaezelandiae* are distinctly separated by their comparatively very small, sublimoniform shape and 4-5(-6), indistinctive longitudinal ridges (Pl. 2: 1).

The review on sequestrate macrofungi in Australia and New Zealand recently published by Lebel & Castellano (1999) clearly indicates that in Australasia the occurrence of many more taxa can be expected in the future. No doubt this data will cast new light upon taxonomy and distribution of "*Gautieria*". As a result, it is still premature to propose final conclusions about the taxonomic status of the taxa at present referred to *Gautieria, Austrogautieria, Protogautieria* (A. H. Smith, 1965) and *Rhodactinia* (Pegler & Young, 1989).

Ectomycorrhiza of Nothofagus with G. inapire

The belowground mycelium of *G. inapire* was found to form a dense mat, enclosing numerous fine roots of *Nothofagus pumilio*. The mycorrhizal nature of the root-fungus association (formation of a compact, fungal mantle of approx. 20 μ m thickness and a paraepidermal Hartig net) could be shown by morpho-anatomical analysis. The identity of the mycobiont was proven by comparing the hyphal features of the mycorrhiza with those of the mycelium attached to the fruitbodies of *G. inpaire*. In addition, DNA fingerprint obtained by PCR/RFLP techniques yielded equal fragments of the ITS1/ITS4 region of fungal nuclear rDNA of both fruitbody and mycorrhiza after restriction with four different endonucleases (Palfner, 2000).

It can be concluded, therefore, that in the South American *Nothofagus* forests *G. inapire* (and its related *G. chilensis*) belongs to the large ecological group of macrofungi forming ectomycorrhiza with *Nothofagus pumilio* (Wright 1988).

The taxonomic position of the genus *Gautieria* within putatively related agaricoid and/or boletoid basidiomycota still remains obscure. Based upon basidiospore morphology and chemical compounds isolated from basidiomata, a possible systematic relationship to Agaricales, Boletales or Cortinariales has been proposed by several authors (Stewart & Trappe, 1985; Hawksworth & al., 1995; Bresinsky, 1996; Molina & al., 1992; Pegler & al., 1993).

By comparing mitochondrial DNA sequences of about 80 genera of Basidiomycetes, Bruns & al. (1998) proposed a close affinity of *Gautieria* to *Gomphus*, *Kavinia* and *Ramaria*. These molecular data have been supported by Agerer (1999) who observed striking morpho-anatomical similarities on and between rhizomorphs and mycelium of *G. inapire*, as compared to analogous structures within basidiocarps of *Gomphus clavatus* and *Ramaria* spp. In addition, ectomycorrhizae of *Gomphus, Kavinia* and *Ramaria* share some distinctive morphological features (Agerer, 1999; Palfner, 2000) that very strongly support the taxonomic position of *Gautieria* within the clade of the Gomphales s.l.

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