# *Ticogloea guttulata* – the first record from oak and the third report world-wide

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Received 16. 6. 2000

**Key words:** Mitosporic fungus, *Ticogloea guttulata*, roots, *Quercus robur*, *Quercus petraea*. – Endophytes, ecology. – Mycobiota of Austria.

**Abstract:** *Ticogloea guttulata*, a mitosporic fungus previously described from roots of *Ticodendron incognitum* from Costa Rica and from a root of *Tilia platyphyllos* in Germany, was isolated several times from roots of *Quercus petraea* and *Q. robur* in Austria. This is the first record of *T. guttulata* from oak and the third report of this fungus world-wide. The species was isolated not only from living but also from dead roots and therefore appears to live endophytically as well as saprophytically. The frequency of colonization of roots by *T. guttulata* was similar for both healthy and diseased oak trees. The presence of *T. guttulata* therefore could not be related to crown defoliation. However, frequency of colonization was influenced by root diameter and soil depth from which roots had been sampled.

**Zusammenfassung:** *Ticogloea guttulata*, ein bisher aus Costa Rica an Wurzeln von *Ticodendron incognitum* sowie aus Deutschland an einer Wurzel von *Tilia platyphyllos* beschriebener mitosporer Pilz, wurde in Österreich mehrmals aus Wurzeln von *Quercus petraea* und *Q. robur* isoliert. Dies ist der Erstnachweis von *T. guttulata* an Eiche und weltweit der dritte Nachweis dieser Art. Die Pilzart wurde sowohl aus Lebendwurzeln als auch aus Totwurzeln isoliert, was auf eine endophytische sowie saprophytische Lebensweise schließen läßt. Wurzeln gesunder und geschädigter Eichen waren ungefähr gleich häufig von *T. guttulata* besiedelt. Die Besiedlungshäufigkeit von Eichenwurzeln durch *T. guttulata* war somit unabhängig vom Kronenzustand. Einen deutlichen Einfluß auf die Besiedlungshäufigkeit hatten hingegen Wurzeldurchmesser und Bodenhorizont (-tiefe), aus dem die Wurzelproben entnommen wurden.

While conducting ecological studies on the mycobiota in roots of healthy and declining oak trees in Austria between 1993-1998 (HALMSCHLAGER & KOWALSKI 1998), more than 80 fungal taxa were obtained from surface-sterilized roots of *Quercus petraea* (MATT.) LIEB and *Quercus robur* L. The microfungal assemblage comprised primarily common soil fungi as well as typical root colonizers, some of which (e.g., *Cryptosporiopsis radicicola* KOWALSKI & BARTNIK) have just recently been described (KOWALSKI & BARTNIK 1995). However, a few taxa did not conform to any other de-

scribed species and therefore prompted further taxonomic investigations which led to the description of the new species *Chalara angustata* KOWALSKI & HALMSCHLAGER (1996) and *Cryptosporiopsis melanigena* KOWALSKI & HALMSCHLAGER (KOWALSKI & al. 1998). Another interesting taxon resembling the genus *Periconia* TODE (ELLIS 1971) was found to match the description of *Ticogloea guttulata* GABRIELE WEBER, F. SPAAIJ & W. GAMS, the type species of a hyphomycete genus described by WEBER & al. (1994). This paper presents the first record of *T. guttulata* from oak and the third report of this fungus world-wide and provides further details on its ecology.

**Material and methods**

Strains of *T. guttulata* were isolated in 1993 and 1994 from oak roots obtained from two sites in Austria (Table 1) that formed part of the interdisciplinary research program on oak decline ("FIW II – Eiche"; HAGER 1993). Site "Patzmannsdorf" (48°36'25"N, 16°18'80"E) is located 40 km north of Vienna in the Weinviertel, site "Niederweiden" (48°12'30"N, 16°54'70"E) is situated in the Marchfeld, about 30 km east of Vienna. According to the classification of the Austrian forest ecoregions (KILIAN & al. 1994), the sites belong to the forest ecoregion 8.1 ("Pannonisches Tief- und Hügelland").

The stand at Patzmannsdorf is a mixed stand of Durmast oak (*Quercus petraea*) and English oak (*Q. robur*) with small-leafed lime (*Tilia cordata* MILL.) and common hazel (*Corylus avellana* L.) as subordinate species. The stand at Niederweiden consists of almost pure English oak with negligible admixture of silver birch (*Betula pendula* ROTH) and wild cherry (*Prunus avium* L.).

At each of the two sites roots were sampled from three healthy-looking[1] and six declining oak trees[2] (seven trees of *Q. petraea* and 11 of *Q. robur*). Excavation and sampling of roots was carried out from eight soil profile pits (depth: 1 m) as well as from additional soil cores (HALMSCHLAGER & KOWALSKI 1998, HALMSCHLAGER 1998). In contrast to sampling from soil profile pits, the latter method confined sampling to 50 cm only.

In advance to the following treatments, all roots were washed thoroughly and were divided into living roots and dead roots. Additionally, living roots were classified into the following root diameter subdivisions (coarse roots included the main roots):

| | | | |
|---|---|---|---|
| very fine roots: | < 2 mm | structural roots: | 11-30 mm |
| fine roots: | 2-10 mm | coarse roots: | > 30 mm |

From each root, a 8 cm long segment was cut and surface-sterilized according to HALMSCHLAGER (1991). Root segments were washed: 1) for one min in ethanol (96%), 2) then five min (three min for very fine roots) in sodium hypochlorite (NaOCl) with 4% available chlorine, and 3) rinsed for 30 sec in ethanol (96%). After surface sterilization was completed, a 5-10 mm piece was cut from the middle of each segment. Each piece was separated into bark and xylem and plated out on the surface of 2% malt extract agar (MEA, 20 g $l^{-1}$ malt extract Difco, 15 g $l^{-1}$ agar Difco) supplemented with 100 mg $l^{-1}$ streptomycin sulfate. Dishes were incubated at room temperature and diffuse daylight. Resulting isolates were transferred to new MEA plates and cultivated at 20 °C in the dark. In total 1357 isolations from living roots and 337 isolations from dead roots wer carried out.

Strains of *T. guttulata* were examined for their morphological and cultural properties on 2% MEA plates, incubated at 20 °C in the dark. Photomicrographs were taken using a Zeiss Axiophot microscope in bright field or interference contrast. One representative strain of *T. guttulata* from *Q. robur* (strain no. 116, CBS 102857) and from *Q. petraea* (strain no. 117, CBS 102858) has been submitted for deposit to CBS Baarn.

[1] Crown thinning classes 1 and 2 according to NEUMANN & POLLANSCHÜTZ (1988)
[2] Crown thinning classes 3 and 4 according to NEUMANN & POLLANSCHÜTZ (1988)

Table 1. Characterization of the study sites (from SCHUME 1992, supplemented by HALMSCHLAGER & KOWALSKI 1998).

| | Patzmannsdorf | Niederweiden |
|---|---|---|
| Altitude (m s. m.) | 310 | 140 |
| Exposure | E-SE | plain |
| Mean temperature (°C)[3] | 9.2 | 9.5 |
| Mean yearly rainfall (mm)[4] | 489 | 495 |
| Soil- and humus-type | Ferric Cambisol with moder-like mull | Chernozem with mull |
| Soil-pH (40-60 cm; $H_2O$) | 5.6 | 8.2 |
| Oak species | *Q. petraea*, *Q. robur* | *Q. robur* |
| Age of stand | 115 | 85 |
| Silvicultural system | Middle forest | High forest |

## Results and discussion

*Ticogloea guttulata* was isolated from roots of *Quercus robur* as well as from roots of *Q. petraea*. Host range therefore has been extended for another two host species. So far the fungus has been recorded only from roots of *Ticodendron incognitum* GÓMEZ-LAURITO & GÓMEZ P. and *Tilia platyphyllos* SCOP. The record from Austria is the third report of this fungus world-wide, since *T. guttulata* was recorded only from Costa Rica and North Germany yet (WEBER & al. 1994).

Moreover, the record from Austria comprised the richest population of *T. guttulata* up to now. In Costa Rica and North Germany, three strains ond one strain were obtained, respectively, the Austrian record comprises 15 isolates[5] (Table 2). Since these isolates were obtained from 13 roots, the two isolates derived from the bark and xylem of the same root may not reflect different individual strains.

### Characters:

Colonies of *T. guttulata* were characterised by slow growth on MEA, reaching a diameter of 27-38 mm after 21 d at 20 °C in the dark. Colonies were mostly flat with sparse aerial mycelium, blackish, with circular or distinctly irregular outline (Colour figs. IV, V).

Vegetative hyphae 2-3.5 µm wide, smooth, lacking chlamydospore-like swellings, with numerous droplets; yellowish, in older cultures yellowish-olive green pigmented.

Conidiogenous cells 5.0-6.0 x 2.7-3.5 µm, arising directly from vegetative hyphae or from short side branches, consisting of 1-3 oval cells, measuring 5-7 x 4-6 µm (Colour fig. V).

Conidia holoblastic, guttuliform, slightly truncate at the base, initially hyaline, later olive brown to blackish, 3.8-5.0 x 2.7-3.5 µm (Figs. 1, 2), forming enormous masses of slimy and tar-like shining droplets on the surface of colonies (Colour figs. IV, VI).

[3] Data from HYDROGRAPHISCHES ZENTRALBÜRO (1998)
[4] Period 1981-1990
[5] Another nine isolates were obtained from declining roots in a following study

A very characteristic colony feature is the yellow-orange discoloration of the agar on the reverse side and at the margin of colonies. The latter can be restricted to a small band surrounding the colony (Colour fig. VI) but sometimes is found within a broader zone from the margin of colony (Colour figs. IV, V). However, after subculturing for 3-5 times and long-term storage (3-5 years) cultures often loose their ability to produce this typical pigmentation, diffusing into the medium. Thus, after five years most of the cultures isolated in 1993 and 1994 are now lacking this typical pigmentation in vitro.

The described cultural and morphological characters of our isolates completely match the description of *Ticogloea guttulata*.

Table 2. Colonization frequency of living and dead oak roots by *Ticogloea guttulata*. (n = number of investigated roots).

| Roots | Bark | Wood | Colonized roots[6] |
|---|---|---|---|
| Living (n = 1357) | 5 (0.4 %) | 7 (0.8 %) | 11 (0.8 %) |
| Dead (n = 337) | 1 (0.3 %) | 2 (0.6 %) | 2 (0.6 %) |

Table 3. Colonization frequency (%) of living oak roots by *Ticogloea guttulata* depending on soil depth and root diameter, respectively (numbers in parentheses refer to no. of sampled roots).

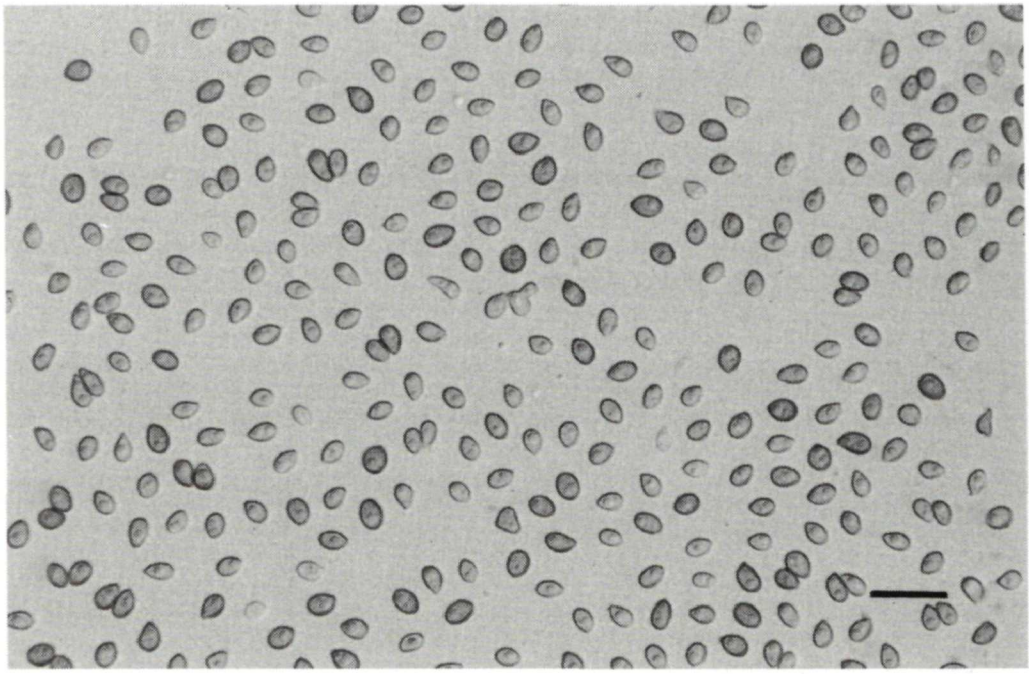
| | Root diam. (mm) | | | | |
|---|---|---|---|---|---|
| Soil depth (cm) | <2 | 2-10 | 10-30 | > 30 | Total |
| 0-50 | 0.2 | 0.4 | 2.7 | 5.9 | 1.0 |
| | (n = 520) | (n = 260) | (n = 149) | (n = 68) | (n = 997) |
| 50-100 | 0.0 | 0.0 | 1.3 | 0.0 | 0.3 |
| | (n = 160) | (n = 80) | (n = 80) | (n = 40) | (n = 360) |
| Total | 0.1 | 0.3 | 2.2 | 3.7 | 0.8 |
| | (n = 680) | (n = 340) | (n =229) | (n = 108) | (n = 1357) |

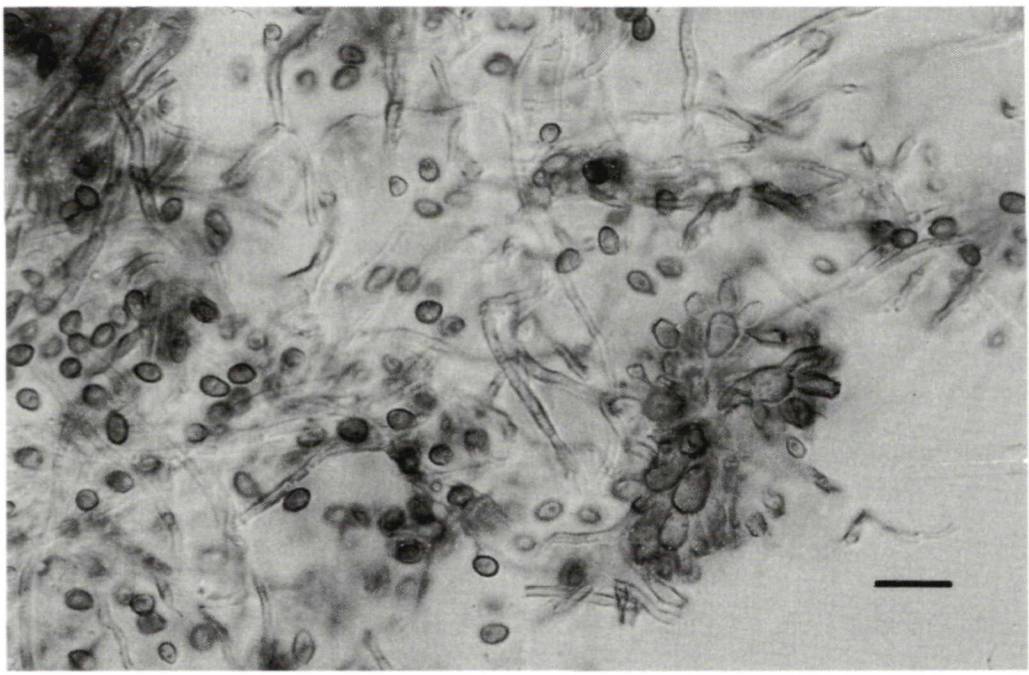
*Ticogloea guttulata* was obtained from both sites (Patzmannsdorf, Niederweiden) investigated in Austria, indicating that the fungus has a broad ecological range (see Table 1). This fact is reflected in the occurrence of this species in the tropics as well as in the temperate zone (WEBER & al. 1994).

While in Costa Rica *T. guttulata* was found in the rhizoplane of *Ticodendron incognitum*, in Austria and in Germany, the fungus was isolated from surface-sterilized roots, where it was found in the bark as well as in the xylem (Table 2). Since the species was isolated not only from living but also from dead roots, it appears to live endophytically as well as saprophytically. The frequency of colonization of roots by *T. guttulata* was similar for both healthy and diseased trees. Therefore, the presence of this species could not be related to crown defoliation. Thus, our first observations did not give any evidence on a potential pathogenicity of *T. guttulata*.

However, frequency of colonization was influenced by root diameter and soil depth (Table 3): colonization frequency of roots from the upper 50 cm soil horizon was three times higher than of roots obtained from 50-100 cm soil depth. Furthermore, colonization of coarse roots by *T. guttulata* was nine times higher compared to roots with a diameter less than 10 mm (fine and very fine roots).

[6] Isolates derived from the bark and xylem of the same root were counted only once.





Figs. 1, 2. *Ticogloea guttulata*. 1. LM of pale to dark-brown, slightly guttuliform conidia. 2. Fragments of hyphae with a dense cluster of conidiogenous cells and ripe conidia (bars: 10 µm).

Since the total number of roots colonized by *T. guttulata* was quite low (0.8 % from 1694 roots investigated), our results can provide some tendencies only. However, they contribute to enhancing our knowledge on the ecology of this little-known species.

It is likely that *T. guttulata* occurs more frequently than expected from the literature because it was attributed to resembling species in genera such as *Exophiala*, *Acarocybe*, *Brachyconidiella*, *Trichobotrytis*, *Periconia*, *Haplobasidion* or *Lacellinopsis* (WEBER & al. 1994) in earlier studies.

This investigation received financial support from the "East-West program" (GZ 45.319/2-IV/6a 93) of the Austrian Ministry of Science. We are grateful to SUSANNE KRUMBÖCK for her excellent technical assistance within this project. Special thanks go also to all colleagues who helped us with the field sampling. Dr KURT GOTTSCHALK (USDA Forest Service, Morgantown, WV) critically read manuscript and improved the language, which is gratefully acknowledged.

**References**

ELLIS, M. B., 1971: Dematiaceous hyphomycetes. – Kew: Commonwealth Mycological Institute.

HAGER, H., 1993: Das Programm zur Erforschung der Eichenerkrankungen in Österreich und einige vorläufige Ergebnisse. – In Bayerische Akademie der Wissenschaften (Herausg.): Zustand und Gefährdung der Laubwälder. Rundgespräche der Kommission für Ökologie **5**: 69-80. – München: Verlag Dr. Friedrich Pfeil.

HALMSCHLAGER, E., 1991: Endophytische Pilze in Blättern, Zweigen und Knospen der Traubeneiche (*Quercus petraea* [MATT.] LIEB.). – Ph.D. thesis, Univ. Bodenkultur Wien.

— 1998: The possible role of *Armillaria* spp. and *Phytophtora* spp. in the oak decline complex. Preliminary results from a three-year survey on two declining oak stands in eastern Austria. – In CECH, T., HARTMANN, G., TOMICZEK, C., (Eds.): Disease/Environment Interactions in Forest Decline, pp. 49-56. – Proc. Internat. IUFRO-Workshop, Federal Forest Research Centre Vienna, March 16-21, 1998, Austria.

HYDROGRAPHISCHES ZENTRALBÜRO (Ed.), 1998: Hydrographischer Jahresbericht von Österreich, Vol. 103/1995. – Wien: Hydrographisches Zentralbüro des BMLF.

KILIAN, W., MÜLLER, F., STARLINGER, F., 1994: Die forstlichen Wuchsgebiete Österreichs. Eine Naturraumgliederung nach waldökologischen Gesichtspunkten (The Forest Ecoregions of Austria. A Landclassification based on Forest Ecology). – Schriftenreihe der Forstlichen Bundesversuchsanstalt Wien, FBVA-Berichte **82**.

KOWALSKI, T., 1998: Inventur der phytopathogenen, endophytischen und saprophytischen Mykoflora im Wurzelbereich gesunder und geschädigter Eichen. – BMWV Research Report, Inst. f. Forstentomol., Forstpathol. und Forstschutz, Univ. Bodenkultur Wien.

— BARTNIK, C., 1995: *Cryptosporiopsis radicicola* sp. nov. from roots of *Quercus robur*. – Mycol. Res. **99**: 663-666.

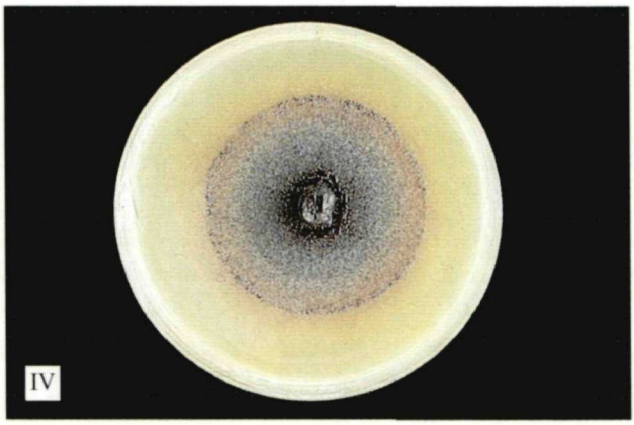
— HALMSCHLAGER, E., 1996: *Chalara angustata* sp. nov. from roots of *Quercus petraea* and *Quercus robur*. – Mycol. Res. **100**: 1112-1116.

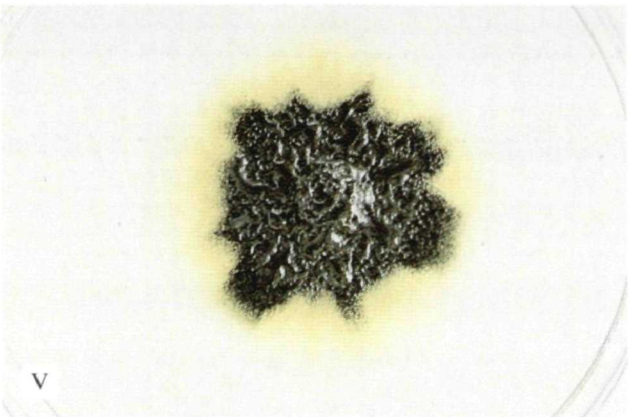
— — SCHRADER, K., 1998: *Cryptosporiopsis melanigena* sp. nov., a root-inhabiting fungus of *Quercus robur* and *Q. petraea*. – Mycol. Res. **102**: 347-354.

NEUMANN, M., POLLANSCHÜTZ, J., 1988: Taxationshilfe für Kronenzustandserhebungen. – Österr. Forstzeitung **99**: 27-37.

SCHUME, H., 1992: Vegetations- und standortkundliche Untersuchungen in Eichenwäldern des nordöstlichen Niederösterreich unter Zuhilfenahme multivariater Methoden. – FIW-Forschungsberichte 1992/3. Herausgeber: Österr. Ges. Waldökosystemforsch. Experiment. Baumforsch. – Univ. Bodenkultur Wien.

WEBER, G., SPAAIJ, F., GAMS, W., 1994: *Ticogloea*, a new genus of hyphomycetes from roots of *Ticodendron incognitum* from Costa Rica. – Mycol. Res. **98**: 660-664.





Colour figs. IV-VI. *Ticogloea guttulata*. IV. Colony with circular outline and typical pigmentation of the medium (after 21 d on MEA at 20 °C in the dark). V. Colony with irregular outline and a broad zone of the characteristic yellow-orange discoloration of the agar at the margin of the colony (after 21 d on MEA at 20 °C in the dark). VI. Colony with distinct slimy and tar-like droplets on the surface of the colony containing masses of conidia. The yellow-orange discoloration of the agar is restricted to a small band surrounding the colony (after 21 d on MEA at 20 °C in the dark).