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Autor: Keller, Siegfried
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Zoophthora giardii Balazy and *Conidiobolus gustafssonii* Balazy (Fungi, Entomophthorales), two entomopathogens new for Switzerland

Siegfried Keller¹

¹ Rheinweg 14, CH-8264 Eschenz, Switzerland

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Corresponding author: Siegfried Keller (siegfried.keller@bluewin.ch)

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Abstract

Meconema meridionale Costa (Orthoptera, Tettigoniidae) infected with *Zoophthora giardii* Balazy (Entomophthorales, Entomophthoraceae) were collected at five localities in the northern half of Switzerland. At one of these sites, the fungus caused epizootics in two subsequent years. Symptoms and morphological data coincide with those given by Balazy who found the fungus on *Meconema thalassinum* De Geer. *Conidiobolus gustafssonii* Balazy (Entomophthorales, Ancylistaceae) was found on a single *Ectobius vittiventris* Costa (Blattodea, Ectobiidae). Symptoms and dimensions of the primary conidia correspond with the original description given by Balazy who found the fungus on *Ectobius lapponicus* L. The two fungi are new for Switzerland and *Meconema meridionale* and *E. vittiventris* represent new hosts for these pathogens.

Key Words

Insect pathogenic fungi, morphology, distribution

Introduction

The Neozygites and the Entomophthorales belong to the phylum Entomophthoromycota of the fungal kingdom. They comprise mainly arthropod-pathogenic species but also members with a saprobiontic life style (Humber 2012).

Recently, two insect species, the southern oak bush-cricket *Meconema meridionale* and the amber wood cockroach *Ectobius vittiventris* were found infected by entomophthoralean fungi in Switzerland. Both insects originate from the mediterranean region but are spreading northwards (T. Haye, pers. comm.). *Ectobius vittiventris* has colonized Germany up to Nordrhein-Westfalen (Schäfer et al. 2016) and is recorded from the Alps up to an altitude of 1400 m (Baur et al. 2004). *Meconema meridionale* has colonized the whole of western Europe up to the North Sea but seems to avoid higher altitudes (<https://www.gbif.org/species/1690429>).

Subsequent microscopic examination of the fungi revealed that they represented members of the genera

Zoophthora and *Conidiobolus* that have not been recorded before in Switzerland. In the present paper, the fungi are described and discussed in detail.

Materials and methods

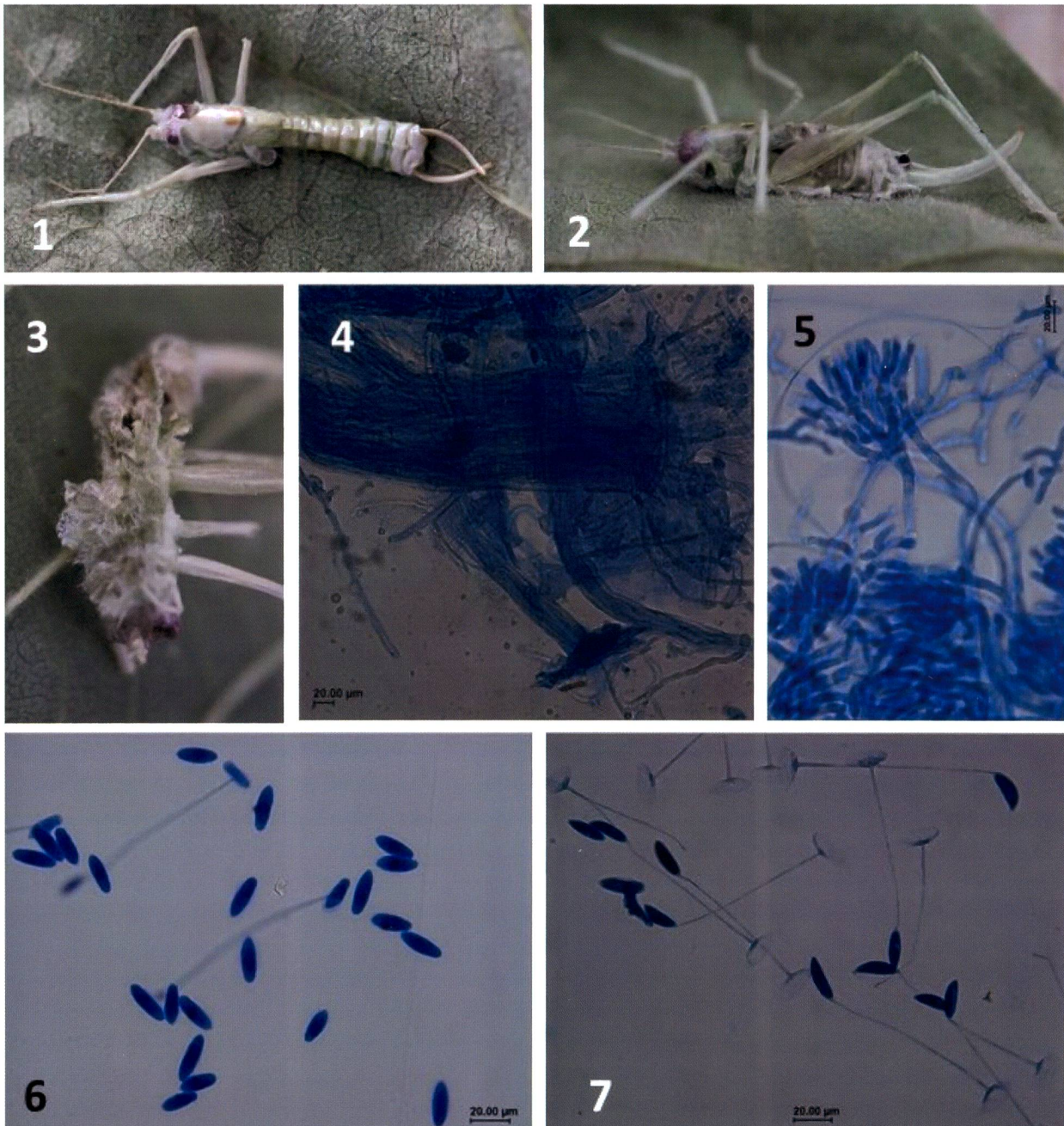
An overview of the collected material is given in Table 1. At Bümpliz BE a striking high number of infected bush crickets were found in a cemetery. Two samples of the material collected on August 2021 were sent to the author. The first one collected on August 12 contained five females and three fragments, the second one collected on August 25 contained three males (Fig. 1), seven females (Fig. 2) and two fragments. All cadavers were found on the underside of leaves of deciduous trees and bushes except those collected at Regensdorf.

A selection of infected *M. meridionale* and the dead *E. vittiventris* were placed individually in small Petri dishes with water. A microscopic slide was placed above the cadavers to collect the projected primary

conidia. Secondary conidia were picked up from the water surface as described by Papierok (2007). Fourteen cadavers of *M. meridionale* and the only cadaver of *E. vittiventris* were carefully dissected into tiny pieces. The fungal material was mounted in lactophenol-cotton blue (LPCB) or in lactophenol-aceto-orcein (LPAO) as described by Keller (1987). Only primary conidia with the axis parallel to the slide and with the whole spore outline in focus, were measured. Primary conidia originating from *E. vittiventris* were obtained by three

methods: 1) by picking up from the leaf; 2) by projection from the cadaver on a slide and 3) by preparation of fungal material from the host tissue.

All measurements were based, if not otherwise stated, on 25 structures per individual host, except cystidia, designated as one series. Usually more than one series was studied from each structure, to assess variation. The number of series is given after the range of the mean values, the range of the extreme values (in brackets) and the ratio length/diameter (L/D).



Figures 1–7. *Zoophthora giardii*. 1. Infected male fixed with rhizoids on a leaf. 2. Infected female. Rhizoids between insect body and leaf are visible. 3. Cadaver from the ventral side showing the layer of rhizoids. 4. Compound rhizoids (LPCB). 5. Branched conidiophores (LPCB). 6. Primary conidia, two with capillary tube (LPCB). 7. Type II secondary conidia or capilliconidia with capillary tubes and remnants of primary conidia (LPCB).

Table 1. Collection data of infected *Meconema meridionale* and *Ectobius vittiventris*. The coordinates were taken from www.map.swisstopo.admin.ch. The collection sites are followed by the official abbreviation of the corresponding Swiss canton.

Host	Collection site with coordinates	Host plant	Collector	Date of collection
<i>Meconema meridionale</i>	Bümpliz BE, 46.94187/7.38426	Catalpa bignonioides	T. Haye	Sept. 17, 2020; Aug. 12, 2021; Aug. 25, 2021
	Muttenz BL 47.53299/7.63401	Catalpa bignonioides	T. Haye	Aug. 18, 2021
	Duggingen BL 47.53207/7.63426	Fagus sylvatica	T. Haye	Aug. 18, 2021
	Eschenz TG 47.65028/8.85586 47.65228/8.87800	Corylus avellana and Prunus sp.	S. Keller	Aug. 27, 2021; Sept. 02, 2021
	Regensdorf ZH (47.43588/8.4619	Ceiling of a house entry	G. Graben-weger	Sept. 09, 2021
<i>Ectobius vittiventris</i>	Bern BE 46.934176/7.431215	Hedera helix	N. Häner	Aug. 25, 2021

Results

The microscopic examination of the *M. meridionale* material revealed the following: The host body was completely filled with hyphae like hyphal bodies. The rhizoids, which fixed the host tightly to the surface consisted of compound hyphae (Fig. 4). The conidiophores were branched (Fig. 5). The endings of the conidiophores, the so-called terminal portions, from which the primary conidia developed, segregated from the conidiophores, before the conidia were formed. The terminal portions were irregularly subcylindrical and measured on average $25.1 \times 5.8 \mu\text{m}$ (Table 2). The primary conidia were subcylindrical and had the widest diameter in the central portion (Fig. 6). The outer wall partly separated from the conidial body. They measured on average $20.0\text{--}21.6 \times 6.8\text{--}7.2 \mu\text{m}$ and had a length/diameter ratio of 2.86–3.08 (Table 2). The indistinct papilla was rounded. The primary conidia germinated either with a capillary germ tube to develop a type II secondary conidium (capilliconidium) or with a short thick germ tube to develop a type I secondary conidium, which resembles the primary conidium. Fully developed type I secondary conidia were too rare to be measured. The capilliconidia were fusiform and slightly bent (Fig. 7). They measured on average $20.1\text{--}20.8 \times 5.7\text{--}6.0 \mu\text{m}$ with a length/diameter ratio of 3.36–3.55 (Table 2). They were formed at the end of a narrow capillary tube which had an average length of $75.1\text{--}92.4 \mu\text{m}$. Usually, a primary conidium formed a single capillary tube but sometimes two or even three capillary tubes were noticed but only one ended with a conidium. Resting spores were not present.

The diseased *Ectobius* was tightly fixed with compound rhizoids on a leaf of *Hedera helix* with the wings slightly opened. The cadaver was surrounded by a white halo of projected conidia (Fig. 8). The mounted material showed different fungal structures. The hyphal bodies, which filled the body cavity, were usually irregularly rounded (Fig. 9), sometimes composed of two, rarely more rounded structures, or irregularly short hyphae-like structures. The conidiophores were unbranched with slightly increased diameter at the end from which a single conidium developed (Fig. 10). The primary conidia were round to slightly pyriform,

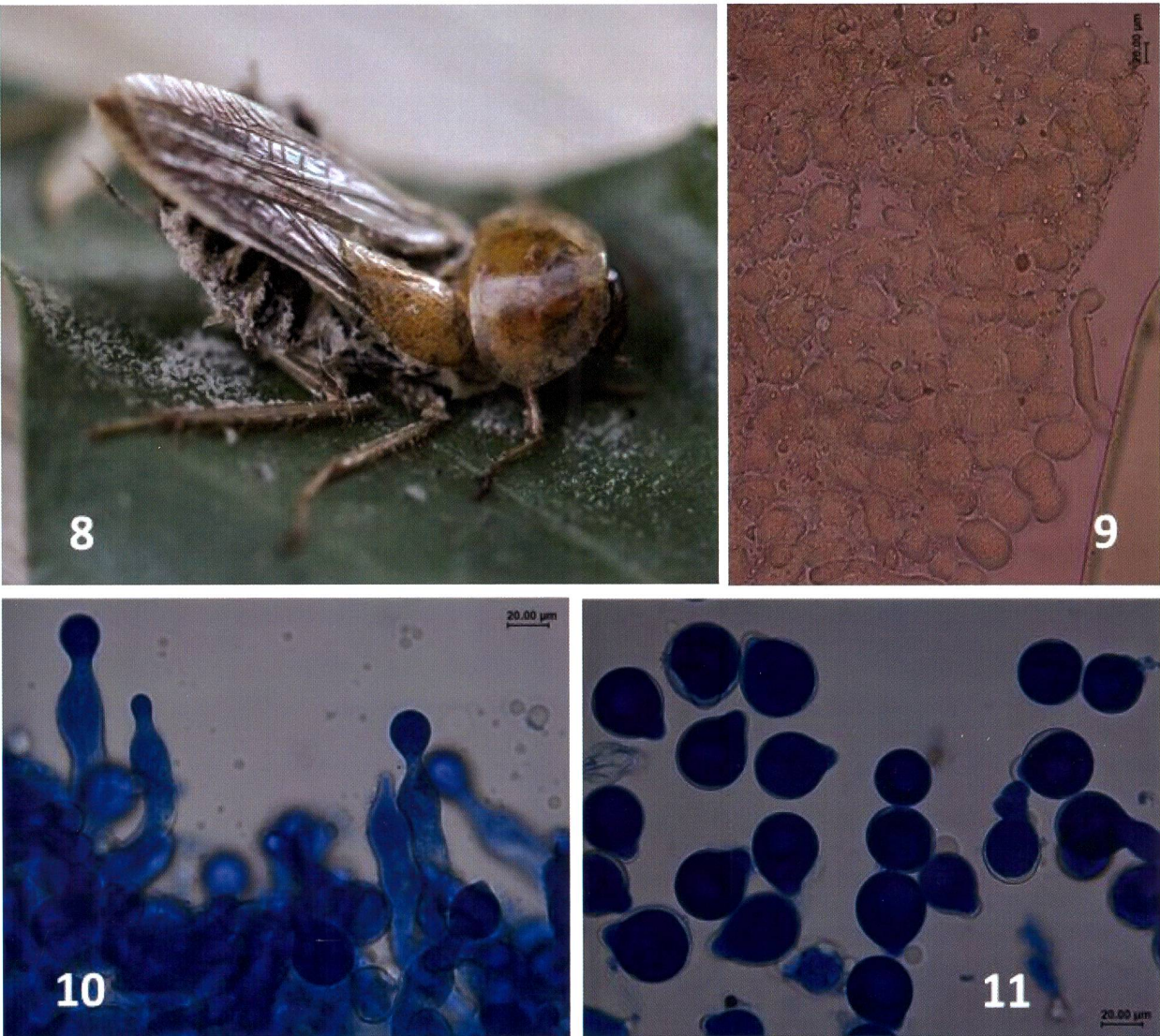
the conidial body smoothly joined the rounded papilla (Fig. 11). Projected conidia measured $43.2 (39\text{--}50) \times 35.6 (31\text{--}40) \mu\text{m}$ with a length/diameter ratio (L/D) of 1.21, conidia taken from the leaf measured $40.6 (37\text{--}46) \times 31.5 (28\text{--}34) \mu\text{m}$, L/D = 1.29, and those from host tissue were $39.9 (37\text{--}44) \times 31.4 (28\text{--}35) \mu\text{m}$, L/D = 1.27. The projected conidia were slightly larger which is probably due to the compression, the other two slides contained either non-fungal material or parts of the host which prevented compression of the conidia. The primary conidia germinate with a single short tube to form a secondary conidium which resembles the primary one. No fully developed secondary conidia were observed. Resting spores were absent.

Meconema meridionale and *E. vittiventris* represent new hosts for the corresponding fungus. Fungal material was deposited at the United Herbaria Zurich under the numbers ZT Myc 66704 and 66705 (*Z. giardii*) and ZT Myc 66706 (*C. gustafssonii*).

Discussion

The description of the fungus attacking *Meconema meridionale* fits quite well the description of *Z. giardii* given by Bałazy (1993). Therefore, we consider the two fungi as identical, although there are minor differences in the length of the capilliconidia. According to the original description, the primary and the capillary conidia measure $19.5\text{--}23.5 \times 6\text{--}7 \mu\text{m}$ and $21\text{--}25 \times 5.5\text{--}6.5 \mu\text{m}$, L/D=3.8 respectively. These small differences could also be the result of different preparation and staining methods.

The finding of *Zoophthora giardii* in Switzerland is not surprising since the species was previously recorded from France, Germany, and Poland (Bałazy 1993) who considered the species as “rather rare”. This is in contrast to the findings in Switzerland, where *Z. giardii* was found at several localities occasionally at an epizootic level. This might be due to another host. Bałazy found the fungus on *Meconema thalassinum* while we found the fungus on *M. meridionale* which represents a new host for *Z. giardii*. It is possible that this species was never in contact with this fungus in the original



Figures 8–11. *Conidiobolus gustafssonii*. **8.** Infected host. Note the white conidia beside the host. **9.** Hyphal bodies (LPAO). **10.** Unbranched conidiophores with developing conidia (LPCB). **11.** Primary conidia (LPCB).

distribution sites, and is therefore more sensitive to fungal infections.

According to the original description (Bałazy 1993) the primary conidia of *C. gustafssonii* measure on average $41\text{--}47 \times 30\text{--}40\text{ }\mu\text{m}$. We measured conidia obtained by three different methods. Those of projected conidia were the largest. However, this may be an artefact, since the conidia become compressed between slide and cover glass during preparation. More accurate data result from measurements of conidia taken from the leaf or host tissue. These preparations contain material which is larger than the conidia and prevent conidia from being compressed. Therefore, we consider $40\text{--}41\text{ (}37\text{--}46\text{)} \times 31.5\text{ (}28\text{--}35\text{)}\text{ }\mu\text{m}$ as the accurate size of the conidia of our material which matches the original description. Bałazy found the fungus on *Ectobius lapponicus*. Our finding on *E. vittiventris* represents a new host of this fungus.

With these findings, the number of entomophthoralean fungi in Switzerland increases to 90 species (Keller 2008). There is no other country with such

Table 2. *Zoophthora giardii*. Dimensions of the fungal structures in μm (PC = primary conidia, SC type II = secondary conidia of the capillary type, Cap. tube = capillary tube, CP = conidiophore, s.d. = standard deviation), based on 25 measurements each.

Structure and slide number	Length (L) (s.d.) min-max	Diameter (D), (s.d.), min-max	Ratio L/D	Stain
PC 20	21.5 (1.14) 20–24	7.2 (0.67) 6–8	3.00	LPCB
PC 24	21.6 (1.31) 19–24	7.0 (0.80) 6–8	3.08	LPCB
PC 29	20.0 (0.87) 19–21	7.0 (0.47) 6–8	2.86	LPCB
PC 30	20.4 (1.31) 19–24	6.8 (0.51) 6–7	3.01	LPCB
PC 34	21.2 (1.25) 19–24	7.2 (0.76) 6–8	2.96	LPCB
SC type II 17	20.1 (0.87) 19–21	5.7 (0.59) 5–7	3.55	LPCB
SC type II 21	20.3 (1.13) 19–22	6.0 (0.39) 6–7	3.36	LPCB
SC type II 24	20.8 (0.96) 19–22	6.0 (0.47) 5–7	3.48	LPCB
Cap. tube 17	76.5 (5.61) 66–85			LPCB
Cap. tube 20	92.4 (8.08) 80–112			LPCB
Cap. tube 24	75.1 (8.00) 61–94			LPCB
CP terminal portion	25.1 (2.22) 21–30	5.8 (0.79) 5–7	4.36	LPAO

a high diversity of classified arthropod-pathogenic Entomophthoromycota. Especially pre-alpine valleys showed a high species richness. More than a third of the species recorded in Switzerland originate from the headwaters of the two rivers Murg (canton Thurgau) and Töss (canton Zurich) (Keller 2008, 2012). These rather undisturbed riverine forests harboured a rich entomofauna which obviously served as hosts of entomophthoralean fungi. The same effect can be expected in alpine ecosystems which are still unexplored regarding insect pathogenic fungi. There will be certainly more species of Entomophthoromycota, not only in Switzerland but worldwide, since this group of fungi has never been subject of specific research, although they play an important role in the regulation of arthropod populations and bear a large potential in pest control.

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