

**Two new species of the genus *Zoophthora* BATKO
(Zygomycetes, Entomophthoraceae):
Z. lanceolata and *Z. crassitunicata***

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Summary. Two new species of the genus *Zoophthora* BATKO are described. *Z. lanceolata* was found on small Diptera. The primary conidia measure $17.7 (\pm 1.3) \times 7.0 (\pm 0.6) \mu\text{m}$ and the capilloconidia $24.4 (\pm 2.1) \times 3.8 (\pm 0.5) \mu\text{m}$; the resting spores have a diameter of $24.0 \pm 1.8 \mu\text{m}$. *Z. crassitunicata* was found on Cantharidae. The primary conidia measure $31.8 (\pm 2.5) \times 9.8 (\pm 0.6) \mu\text{m}$ and the capilloconidia $33-39 \times 8-9 \mu\text{m}$. The resting spores have a diameter of $43.5 (\pm 3.0) \mu\text{m}$ including the brown episporium.

Introduction

During summer 1978, three small fungus-infected Diptera and ten dead Coleoptera were collected on the bank of a brook within a forest near Rickenbach ZH, Switzerland. It was assumed that the death of the insects was caused by entomophthorosis. The examination of the material showed the presence of two species of the genus *Zoophthora* BATKO (1964) sensu REMAUDIÈRE & HENNEBERT (1980), which however could not be attributed to an existing taxon. Therefore two new species are proposed and described hereafter.

Methods

The methods for the preparation of the conidia and resting spores, for the histological examination of the infected hosts and for the isolation of the fungi were described in an earlier paper (KELLER, 1978). All measurements are based on preparations mounted in lactophenol-cottonblue (LPCB).

Description of the New Taxa

1. *Zoophthora lanceolata* KELLER sp. nov. (Plate 1, figs. 1—7)

Conidia primaria $15-24 \times 6-9 \mu\text{m}$, subcylindrica vel ellipsoidea, papilla conica instructa, membrana externa abruptens, uninucleata. Conidia secundaria $21-30 \times 3.5-5 \mu\text{m}$, fusiformes usque ad subfalcata, hyphis capillaribus evoluta. Sporae $19-29 \mu\text{m}$, globosae, hyalinae, leves. Conidiophora ramosa, rhizoma adsunt. Ad diptera diversa. Helvetia. Typus ZT, cotypi K, BPI.

Primary conidia $15-24 \times 6-9 \mu\text{m}$ (av. $17.7 \times 7.0 \mu\text{m}$), subcylindrical, oblong ovoid to ellipsoid, symmetrical; papilla conical,

demarcated from the body of the conidium by a collar; uninucleate; outer membrane separated from the body of the conidium. Secondary conidia $15-32 \times 3-5 \mu\text{m}$ (av. $24.4 \times 3.8 \mu\text{m}$) formed at the end of capillaries $40-80 \mu\text{m}$ long, slender, fusiform, slightly curved. Resting spores $19-29 \mu\text{m}$ (av. $24.0 \mu\text{m}$), spherical, hyaline, smooth. Conidiophores branched, rhizoids present, cystidia not observed. Parasite of small Diptera.

Localities and sampling dates: *Switzerland*, ZH, Rickenbach: One infected Diptera collected on each of three dates, June 12, July 3 and August 21, 1978. — *France*, La Giétaz: One specimen (Brachycera) collected on October 9, 1977, leg. REMAUDIÈRE.

The specimens collected in Switzerland were found in a forest on the bank of a brook attached to the underside of leaves of *Aegopodium podagraria* L., the specimen from France was attached to a leaf of *Acer* sp.

The holotype of *Z. lanceolata* is represented by insect nr. D780821: Slide containing conidia in LPCB and slides containing histological sections through infected insect.

Symptoms: Infected insects attached to substrate by rhizoids and partially covered with white to greyish mycelium. Hyphal bodies hyphae-like, short, branched or unbranched, multinucleate. Conidiophores branched (fig. 5), cystidia not observed.

Conidia: Primary conidia subcylindrical, oblong ovoid to ellipsoid, symmetrical, largest diameter in the apical half (figs. 1 and 2). Papilla conical, usually rounded, demarcated from the body of the conidium by a collar. Outer membrane separated from the body of the conidium. Cytoplasm containing one nucleus with one or two lateral, prominent vacuoles. Primary conidia $15-24 \times 6-9 \mu\text{m}$, average $17.7 \times 7.0 \mu\text{m}$; ratio length/diameter 2.4—2.6. Detailed data are listed in tab. 1.

Secondary conidia (capilloconidia) formed at the end of capillaries measuring $40-80 \mu\text{m}$, slender, fusiform and slightly curved (figs. 3 and 4), largest diameter in the basal half. Often one or two vacuoles adjacent to the nucleus. Capilloconidia $15-32 \times 3-5 \mu\text{m}$, average $24.4 \times 3.8 \mu\text{m}$; ratio length/diameter 6.1—6.9 (tab. 1).

Resting spores: One of the four cadavers contained resting spores of different degrees of maturity (figs. 6 and 7). Mature resting spores $19-29 \mu\text{m}$, average $24.0 \mu\text{m}$, spherical, hyaline, smooth (tab. 1).

Culture: *Z. lanceolata* was isolated from two insects (culture nrs. 95 and 115). The fungus grows rapidly on coagulated egg yolk diluted with milk (EYM), on Sabouraud dextrose-agar enriched with egg yolk (SDAEY) and on Sabouraud dextrose-agar (SDA). On SDAEY the colonies reach a diameter of 20—30 mm after 8 days at 20° C, and 70—80 mm after 16 days. The mycelium has a light

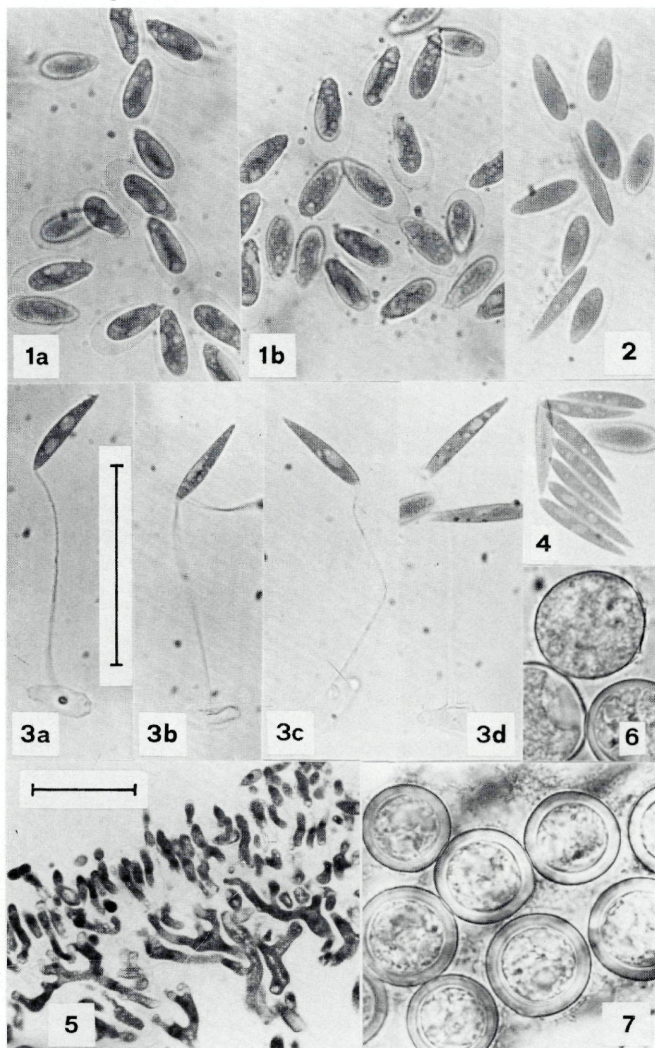


Plate 1, figs. 1—7: *Zoophthora lanceolata* KELLER. 1. Primary conidia showing separated outer membrane (LPCB). — 2. Primary conidia and two secondary conidia (capilloconidia) (LPCB). — 3. Capilloconidia (LPCB). — 4. Capilloconidia detached from the capillary tube (LPCB). — 5. Histological section through infected insect showing ramified conidiophores (haematoxylin). — 6. Immature resting spores (LPCB). — 7. Mature resting spores (LPCB). Bar in figs. 1 and 5 represents 50 μ m, same magnification of figs. 1—4, 6, 7

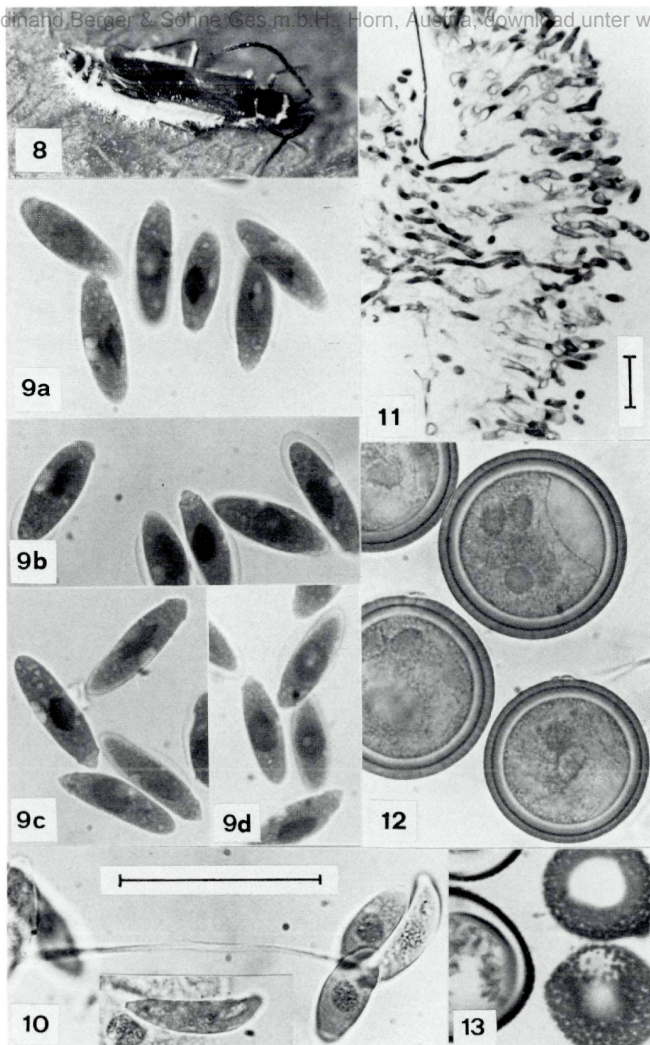


Plate 2, figs. 8–13: *Zoophthora crassitunicata* KELLER. 8. Infected beetle (ca. $6\times$ nat. size). — 9. Primary conidia. Note that the outer membrane usually separates only partially from the body of the conidium (LPCB). — 10. Secondary conidium (capilloconidium). Inset: Capilloconidium detached from the capillary tube (LPCB). — 11. Histological section through infected beetle showing the usually unbranched conidiophores (haematoxilin). — 12. Resting spores. Note the hyaline spore wall surrounded by the brown episporium (LPCB). — 13. Histological section through resting spores. The almost tangential sections on the right show the regular, fine knobs on the surface of the episporium (haematoxilin). Bar in figs. 10 and 11 represents $50\ \mu\text{m}$, same magnification of figs. 9, 10, 12, 13

Table 1. Dimensions (in μm) of primary conidia, capilloconidia, and resting spores from insects and from cultures of *Zoophthora lanceolata* KELLER, based on 50 measurements each

	Origin	Nr.	Length l		Diameter d		l/d
			\bar{x} (s_x)	Min—Max	\bar{x} (s_x)	Min—Max	
Primary conidia	Insect	3	17.1 (1.1)	15—19	6.6 (0.7)	6 — 9	2.6
		6	17.2 (1.2)	15—19	6.9 (0.7)	6 — 9	2.5
		10	17.6 (1.6)	15—24	7.2 (0.5)	6 — 9	2.4
	Culture	GR	18.9 (1.3)	16—23	7.4 (0.6)	6 — 9	2.6
		95	22.4 (2.0)	18—28	8.1 (0.7)	7 — 10	2.8
Capilloconidia	Insect	115	20.2 (2.0)	17—27	7.0 (0.6)	6 — 9	2.9
		2	26.1 (1.8)	23—30	3.8 (0.5)	3.5— 5	6.9
		7	24.3 (1.7)	21—28	4.0 (0.6)	3.5— 5	6.1
		11	26.3 (1.7)	22—30	3.8 (0.4)	3.5— 5	6.9
	Culture	GR	20.9 (3.0)	15—32	3.4 (0.3)	3 — 4	6.2
		95	31.8 (2.7)	25—36	4.8 (0.4)	3.5— 6	6.6
Resting spores	Insect	115	29.6 (4.3)	23—40	4.4 (0.6)	3.5— 6	6.7
		GR			24.0 (1.8)	19 — 29	

grey colour and the colonised medium is slightly darkened. No resting spores form in vitro; the conidia are slightly larger than those from insects (tab. 1).

Distinguishing characters: The species has the characters typical of the genus *Zoophthora* BATKO sensu REMAUDIÈRE & HENNEBERT (l.c.). It is very similar to *Z. radicans* (BREFELD) BATKO (l.c.) (= *Empusa sphaeosperma* sensu THAXTER, 1888), especially with regard to the primary conidia and the resting spores. However, a clear differentiation between the two species is possible on the basis of their capilloconidia. Those of *Z. radicans* measure 13.3—

Table 2. Dimensions (in μm) of primary conidia and resting spores (including episporium) from *Zoophthora crassitunicata* KELLER, based on 50 measurements each

	Nr.	Length l		Diameter d		l/d
		\bar{x} (s_x)	Min—Max	\bar{x} (s_x)	Min—Max	
Primary conidia	3	31.4 (2.7)	25—36	9.8 (0.6)	8.5—11	3.2
	7	32.1 (2.2)	27—36	9.8 (0.6)	8.5—12	3.3
Resting spores	14			43.9 (3.4)	36 — 53	
	15			44.2 (3.1)	36 — 56	
	16			44.7 (3.1)	36 — 51	
	17			43.2 (2.8)	39 — 49	
	18			44.0 (2.9)	39 — 48	
	20			41.2 (2.6)	35 — 47	

16.3×5.0—5.4 μm (REMAUDIÈRE et al., 1976), whereas those of *Z. lanceolata* are 20.9—26.3×3.4—4.0 μm ; the ratio length/diameter of *Z. radicans* is 2.7—3.1, while that of *Z. lanceolata* is 6.1—6.9 respectively.

Name: The name "*lanceolata*" characterises the shape of the capilloconidia.

Holotype: ZT, leg. KELLER. Paratypes: Deposited in the following herbaria: ZT, K, BPI.

Type host: Diptera.

Type locality: Switzerland, Rickenbach, ZH.

Cultures: Deposited at CMI and CBS.

2. *Zoophthora crassitunicata* KELLER sp. nov. (Plate 2, figs. 8—13)

Conidia primaria 25—36×8.5—12 μm , subcylindrica vel subfusiformes, papilla conica praedita, membrana externa abruptens, uninucleata. Conidia secundaria 33—39×8—9 μm , fusiformes vel falciformes, hyphis capillaribus evoluta. Sporae 35—56 μm , globosae, membrana crassa brunnea instructae. Rhizoma adsunt. Ad Coleoptera (Cantharidae). Helvetia. Typus ZT, cotypi K, BPI.

Primary conidia 25—36×8.5—12 μm (av. 31.8×9.8 μm), subcylindrical to slightly fusiform, usually symmetrical; papilla conical, demarcated from the body of the conidium by a collar; outer membrane partially or completely separated from the body of the conidium; uninucleate. Capilloconidia 33—39×8—9 μm , fusiform to falciform, slightly curved. Resting spores spherical, 35—56 μm (av. 43.5 μm) including the closely enveloping, brown episporium. Rhizoids present. Parasite of Cantharidae (Coleoptera).

Localities and sampling dates: *Switzerland*, ZH, Rickenbach: Two infected beetles were collected on June 5 and eight more on June 12, 1978. All specimens were found in a forest on the bank of a brook on the underside of leaves of *Aegopodium podagraria* L.

The holotype of *Z. crassitunicata* is represented by insect nr. C780605/1: Slide containing conidia in LPCB and slides containing histological sections through infected insect.

Symptoms: Conidia formed on the two specimens collected on June 5. In one case, the white conidiophore bands along the intersegmental membrane of the abdomen and between head and prothorax were already visible at the moment of the collection (fig. 8). Both cadavers were fixed and examined histologically. The beetles collected on June 12 contained resting spores and did not produce conidia in the humidity chamber. All cadavers were attached by rhizoids.

Conidiophores: In the histological preparations the conidiophores appear usually unbranched (fig. 11).

Conidia: Primary conidia subcylindrical to slightly ellipsoid, usually symmetrical; outer membrane usually apically, often laterally

Table 3. Comparison of *Zoophthora crassitunicata* KELLER with morphologically similar species

Species	Primary conidia		Capilloconidia		Resting spores			Host	Author(s)
	Length l × Diameter d	l/d	Length l × Diameter d	l/d	Diameter	Episporium			
<i>Z. crassitunicata</i>	31–32 × 10	3.2–3.3	33–39 × 8–9	4.2	41–45	present	Cantharidae (Coleoptera)		
<i>Z. elateridiphaga</i>	28–31 × 9–11	2.5–3.4	31–33 × 7	4.6–4.8	31–38	absent	<i>Agriotes sputator</i> (Coleoptera)	KELLER, unpubl.	
<i>Z. occidentalis</i>	35 × 10	3.5	?		20–35	absent	A species of aphids (Homoptera)	THAXTER, 1888	
<i>Z. aphidis</i>	27–30 × 10–12	2.5–2.7	20–23 × 9–10	2.0–2.4	36–41	present	<i>Anoecia corni</i> (Homoptera)	KELLER, unpubl.	
<i>Z. phalloides</i>	30–34 × 7–8	4.2–4.5	21–23 × 6–8	2.8–3.4	?	?	Aphididae (Homoptera)	REMAUDIÈRE et al., 1976	

or completely separated from the body of the conidium; uninucleate, nuclei large, spherical to ellipsoid, measuring $8 \times 8-12 \mu\text{m}$ on average. Papilla conical, demarcated from the body of the conidium by a collar. The primary conidia measure $25-36 \times 8.5-12 \mu\text{m}$, average $31.8 \times 9.8 \mu\text{m}$, the ratio length/diameter being 3.2-3.3. Detailed data are listed in tab. 2.

The secondary conidia are formed at the end of capillary tubes. They are fusiform to falciform, curved, the inner side of the curve being slightly concave (fig. 10). They measure $33-39 \times 8-9 \mu\text{m}$, the ratio length/diameter being 4.2 (only 4 capilloconidia observed).

Resting spores: The resting spores are spherical and double walled (fig. 12). The inner wall is hyaline and closely enveloped by a brown episporium. In preparations mounted in LPCB the episporium never separated from the hyaline wall, but it did so upon sectioning. In LPCB-preparations, the episporium looks smooth at a magnification of 400 times. However histological examinations showed that the outside is covered densely and regularly with minute knobs (figs. 13). The resting spores have a diameter of $35-56 \mu\text{m}$, average $43.5 \mu\text{m}$. The majority are obviously binucleate, but higher numbers of nuclei (maximum 8) were observed in some.

Culture: Attempts to isolate the fungus failed.

Distinguishing characters: *Z. crassitunicata* has primary and secondary conidia typical for the genus *Zoophthora* BATKO sensu REMAUDIÈRE & HENNEBERT (l.c.), but the closely enveloping episporium of its resting spores is unknown from other species. The present species has to be differentiated from *Z. elateridiphaga* TURIAN, *Z. occidentalis* THAXTER, *Z. aphidis* (HOFFM. in FRES.) BATKO sensu REMAUDIÈRE & HENNEBERT (l.c.) [non sensu THAXTER] and *Z. phalloides* BATKO. Some evidence for the differentiation is given by the host-specificity. *Z. elateridiphaga* is hitherto known only from *Agriotes sputator*, the other three species from a few aphid species. A clear differentiation however has to be based primarily on morphological characters. The comparison in tab. 3 shows that *Z. crassitunicata* differs from *Z. elateridiphaga* and *Z. occidentalis* mainly in the morphology of the resting spores, and from *Z. aphidis* and *Z. phalloides* mainly in the shape and dimensions of the primary conidia and/or capilloconidia.

Name: The name "*crassitunicata*" characterises thick-walled membrane of the resting spores.

Holotype: ZT, leg. KELLER; Paratypes: Deposited in the following herbaria: ZT, K, BPI.

Type host: Cantharidae (Coleoptera), probably *Malthodes* sp. (C. LIENHARD, pers. comm.).

Type locality: Switzerland, Rickenbach, ZH.

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