Entomophthora gigantea sp. nov. and *E. caroliniana* (THAXTER) comb. nov., two pathogens of *Tipula paludosa* MEIG.

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Summary. *Empusa caroliniana* THAXTER is redescribed and transferred to the genus *Entomophthora*. The primary conidia are oblong ovoid to long ellipsoid, 33.6 ± 3.2 µm × 14.2 ± 1.4 µm. The species forms to variants of secondary conidia of the adhesive type. The pyriform variant has mean dimensions of 24.9 ± 2.3 µm × 14.5 ± 1.5 µm, those of the fusiform one 38.9 ± 3.4 µm × 9.2 ± 0.8 µm. The resting spores are spherical, hyaline and smooth, 37.0 ± 3.7 µm. Rhizoids and cystidia are not present.

Entomophthora arrenoctona GIARD is considered to be a synonym.

The primary conidia of *Entomophthora gigantea* sp. nov. are spherical, 92.2 ± 8.8 µm × 76.6 ± 8.6 µm. The secondary conidia are slightly smaller but of similar shape. The resting spores are spherical, hyaline and smooth, 62.6 ± 6.4 µm. The conidiophores are simple; rhizoids and cystidia absent.

Introduction

In previous years epizootics greatly decreased populations of adult *Tipula paludosa* MEIG. in the north-eastern region of Switzerland. They were caused by two species of *Entomophthora* (KELLER, 1977). It was assumed that the dominat species was indentical to *E. arrenoctona* GIARD (1888), but it also possessed some characteristics of *Empusa caroliniana* THAXTER (1888). The other species, at that time known only from mixed infections with that previously mentioned, has not been described before. Since 1976 additional fungal material has been collected allowing the identity of the two fungi to be clarified.

Methods

To induce the fungus to sporulate, the air-dried cadavers of *T. paludosa* were individually placed in small Petri-dishes containing water. Projected primary conidia were collected on a slide placed above the specimen. Most of these primary conidia were mounted in lactophenol-cottonblue (LPCB) on the slide, the rest were left to produce secondary conidia, which were collected on a second slide placed above them. After 8—16 hours the cadavers were transfered to 70% ethanol and after dissection mounted in LPCB. All measurements were made from LPCB-preparations.

For histological examination, the cadavers stored in ethanol were fixed in Bouin's fixative, embedded in plastic embedding medium (JB4), sectioned in 5 μm sections and stained with a modified haematoxilin-stain. Nuclei of primary conidia were stained with the Feulgen reaction stain.

Two media were used for isolations: (1) egg yolk diluted with milk 1 : 1 (EYM) and coagulated at 80° C for 70 minutes and (2) Sabouraud dextrose-agar enriched with egg yolk (1 egg yolk per 200 ml Sabouraud-agar) (SDAEY). For the isolation experiments, projected conidia were collected on a sterile slide, removed with a piece of medium and inoculated into the culture tube.

Results

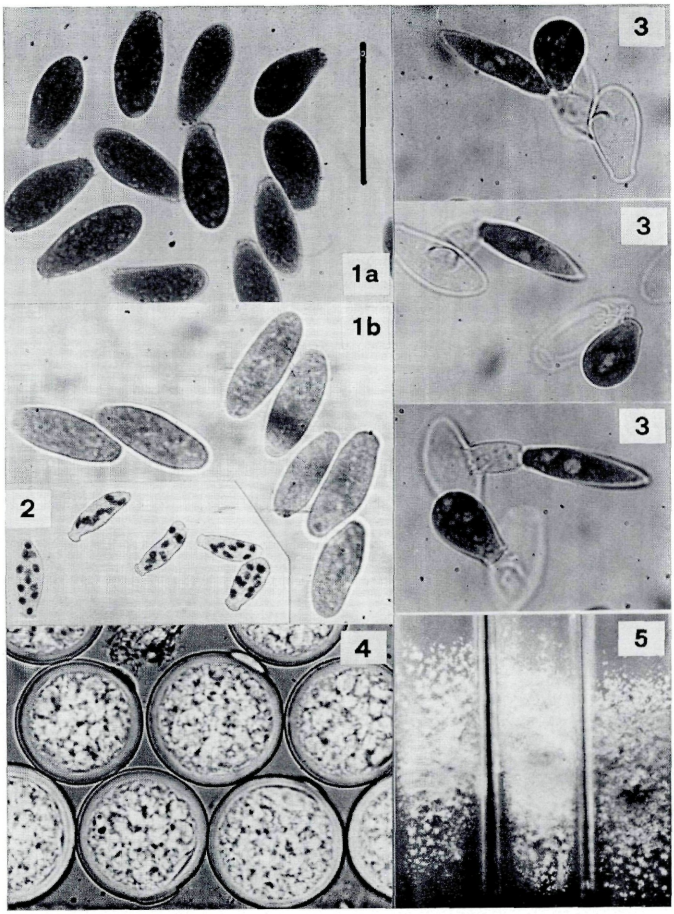
Entomophthora caroliniana (THAXTER) KELLER comb. nov. (plate 1, figs. 1—5)

Basionym: *Empusa caroliniana* THAXTER, Mem. Boston Soc. Nat. Hist. 4, 167, 1888 (April).

Synonym: *Entomophthora arrenoctona* GIARD, Bull. sci. France et Belge 19 (Sér. 3, Vol. 1), 305, 1888 (July).

In a previous paper, KELLER (1977) described an entomophthorosis, which regularly occured in various regions of North-East Switzerland and which caused mortalities up to 80% of the imaginal populations of *Tipula paludosa*. The great majority of the cadavers were males. Then it was assumed that this fungus is identical with *Entomophthora arrenoctona* GIARD. This opinion was subsequently supported by the fact, that this fungus was rediscovered near GIARD's type locality (G. REMAUDIÈRE, pers. comm.). On the other hand, KELLER (l. c.) also mentioned the presence of some primary conidia of the epapillata-type (LAKON, 1919), which so far are known only from *Empusa caroliniana* THAXTER. Furthermore, the dimensions of the conidia correspond with the ones reported for this fungus. However, this identification was questionable because the shape of the secondary conidia and the dimensions of the resting spores failed to correspond with those in THAXTER's description.

In autumn 1977 fresh material was collected and examined especially with regard to this characters. The great majority proved to be infected by the *E. arrenoctona*-type. However, in one population material was observed which closely matched the description of *E. caroliniana*, especially shape and dimensions of the primary conidia were similar. This population also contained many primary conidia transitional in shape between the two types. However, all of them produced identical secondary conidia. Moreover cultures isolated from conidia of the *E. arrenoctona*-type grew identically to those isolated from the *E. caroliniana*-type conidia. Therefore it must be



Entomophthora caroliniana (figs. 1—5). 1. Primary conidia showing typical shape (a) and epapillata variant (b). 2. Primary conidia with Feulgen-stained nuclei. 3. The two variants of secondary conidia of the adhesive type. 4. Resting spores. 5. Four-week old cultures on Sabouraud dextrose-agar. (Bar in fig. 1a represents 40 μm — figs. 1, 3 and 4, same magnification)

concluded, that *E. arrenoctona* GIARD is conspecific with *E. caroliniana* THAXTER. Finally the re-examination of THAXTER's collections confirmed our suspicion (R. A. HUMBER, pers. comm.).

Primary conidia, in accordance with THAXTER's description, oblong ovoid to long ellipsoid, often asymmetric, normally containing 6—10 nuclei (fig. 2). Papilla weakly developed and sometimes hardly distuinguishable from the apex (fig. 1). They measure 22—51 µm × 11—19 µm, average 33.6±3.2 µm × 14.2±1.4 µm, the ratio length/diameter (l/d) being 2.36. The dimensions given by THAXTER are 15—45 µm × 10—26 µm, average 37 × 14 µm. In contrast to THAXTER's description, two variants of secondary conidia of the adhesive type are formed (fig. 3). The pyriform variant measures 18—33 µm × 10—24 µm, average 24.9±2.3 µm × 14.5±1.5 µm (l/d = 1.72), the fusiform one 30—55 µm × 7—13 µm, average 38.9±3.4 µm × 9.2±0.8 µm (l/d = 4.23). The germ tube of the former has a mean length of about 15 µm, that of the latter about 35 µm. The resting spores are spherical, hyaline and smooth (fig. 4). They have a diameter of 24—55 µm, average 37.0±3.7 µm. These dimensions differ slightly from those of THAXTER, who reported the diameter as 37—55 µm, average 45 µm. Conidiophores simple; rhizoids and cystidia absent. Detailed data are listed in tab. 1. Fungal material is deposited at the Institut für Spezielle Botanik, Eidgenössische Technische Hochschule, Zürich, Switzerland (ZT).

The species was isolated on several occasions. On SDAEY the colonies reach a mean diameter of about 5 cm after 4 weeks at 20° C. The fungus grows even slowlier on EYM and on Sabouraud dextrose-agar. The mycelium of 4 weeks old cultures on SDAEY is white to light brown, velvet-like or fluffy, sometimes folded (fig. 5). Cultures are deposited at the Commonwealth Mycological Institute, Ferry Lane, Richmond, Surrey, Great Britain, and at the Centraalbureau voor Schimmelcultures, Baarn, Netherlands.

Entomophthora gigantea KELLER sp. nov.

Conidia primaria sphaerica, papilla conica instructa, 57—123 µm × 46—105 µm (medio 92.2±8.8 µm × 76.6±8.6 µm), vacuolis numerosis impleta, ⩾50 nucleis observata. Conidia secundaria habitu primariis similia, 76.2±6.2 µm × 65.7±6.4 µm. Sporae sphaericae, hyalinae, leves, 48—93 µm (medio 62.6±6.4 µm). Conidiophora simplices, rhizoma et cystidia nulla. Ad *Tipulam paludosam* (MEIG.). Helvetia. Typus ZT. Cotypi CMI, CBS.

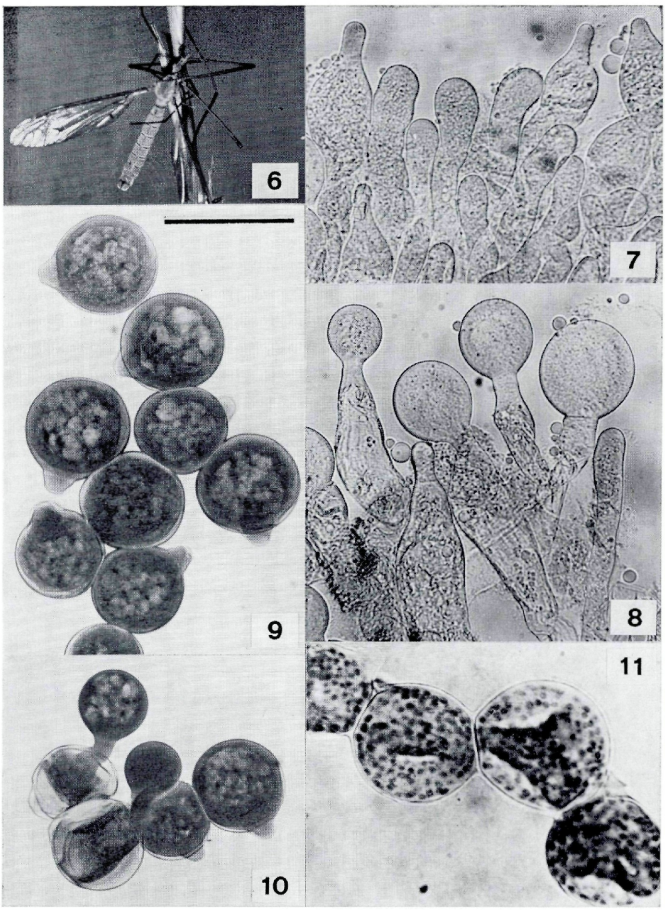
Primary conidia with spherical body, papilla conical, rounded, 57—123 µm × 46—105 µm (av. 92.2±8.8 µm × 76.6±8.6 µm), numerous vacuoles, more than about 50 nuclei. Secondary conidia like the primary, 76.2±6.2 µm × 65.7±6.4 µm. Resting spores spherical, hyaline and smooth, 48—93 µm (av. 62.6±6.4 µm). Conidiophores simple, neither rhizoids nor cystidia.

Table 1. Dimensions (in μm) of primary conidia, secondary conidia and resting spores of *E. caroliniana* from *T. paludosa*, based on 50 measurements per individual

	Ind.	Lenght l			Diameter d			l/d
	no.	$\bar{x}$	($\pm s_{\bar{x}}$)	min-max	$\bar{x}$	($\pm s_{\bar{x}}$)	min-max	
Primary conidia	16	30.1	(3.4)	23—36	14.9	(1.8)	12—18	2.0
	18	27.7	(3.5)	22—39	14.3	(1.9)	12—19	1.9
	31	42.6	(3.8)	36—51	15.2	(1.4)	13—18	2.8
	48	32.5	(2.8)	24—38	14.4	(1.0)	12—16	2.2
	98	35.6	(2.7)	29—40	13.1	(1.3)	11—16	2.7
	118	33.3	(2.7)	27—39	13.0	(1.1)	11—16	2.6
Secondary conidia								
pyriform	30	23.1	(2.7)	19—30	12.6	(1.4)	10—17	1.8
	39	24.6	(2.3)	21—29	14.2	(1.1)	12—17	1.7
	42	28.6	(2.3)	24—33	18.4	(2.1)	15—24	1.6
	48	27.1	(2.3)	22—33	15.4	(1.5)	12—19	1.8
	118	21.3	(1.8)	18—25	11.9	(1.2)	10—15	1.8
fusiform	38	41.4	(2.7)	36—47	9.2	(0.8)	9—11	4.5
	41	38.4	(3.6)	36—47	8.7	(0.7)	7—10	4.4
	44	36.8	(3.3)	30—45	9.9	(0.7)	9—12	3.7
	45	35.2	(2.6)	30—42	8.7	(0.8)	7—11	4.1
	48	42.5	(4.6)	33—55	9.4	(1.1)	7—13	4.5
Resting spores	85				33.6	(2.8)	28—41	
	86				38.7	(2.8)	29—45	
	87				38.6	(3.6)	28—48	
	88				37.6	(3.4)	32—46	
	89				35.7	(2.9)	25—41	
	91				37.7	(6.9)	24—55	

Localities and sampling dates: Infected tipulids were collected from the following sites in Switzerland: (1) Nussbaumen TG: a single male on August 25, 1976; (2) Alterwilen TG: 5 specimens on September 12, 1976, and 6 specimens, 5 males and 1 female, on September 8, 1977; (3) Zürich-Reckenholz: 4 specimens, 3 males and 1 female, on September 6, 1977.

Symptoms: All cadavers of *T. paludosa* were collected in meadows, either close to forests, or in open fields. They were usually attached to tall Gramineae, in a position observed in that of living animals, except that their long legs clasped the plants closely (fig. 6). No external signs indicated the presence of the fungus; there were no rhizoids. The hyphal bodies are usually a conspicuous, bizarre, amoeboid shape (figs. 12, 14 and 17), but there are also hyphae-like ones (fig. 13). The conidiophores have a mean diameter of 15—20 μm; they are simple and terminally enlarged (fig. 7) and form at the thin-walled parts of the insect, predominantly on the thorax. The primary conidia develop from the terminal swellings (fig. 8); the first are formed and projected within 6—8 hours after the dry cadavers are moistened at 20° C. Cystidia were not observed.



Entomophthora gigantea (figs. 6—11). 6. Infected *Tipula paludosa* (ca. 1.5× nat. size). 7. Conidiophores with primary conidia beginning to form. 8. Formation of primary conidia. 9. Primary conidia. 10. Formation of secondary conidia. 11. Primary conidia with Feulgen-stained nuclei. (Bar in fig. 9 represents 100 μm — figs. 7—11, same magnification)

Conidia: The primary conidia have a spherical body (fig. 9). They contain numerous vacuoles and more than about 50 nuclei (fig. 11). The conical, rounded papilla is clearly demarcated from the body of the conidium. The colidia measure 57—123 µm × 46—105 µm, average 92.2±8.8 µm × 76.6±8.6 µm. Detailed data are listed in tab. 2. The secondary conidia are formed at the end of a short germ tube. They resemble the primary ones (fig. 10) and measure 65—88 µm × 57—76 µm, average 76.2±6.1 µm × 65.7±5.6 µm (tab. 2).

Resting spores: Some of the cadavers contained resting spores. They develop from the amoeboid hyphal bodies (figs. 14 and 18) or terminally on the hyphae-like hyphal bodies (fig. 13). They are spherical hyaline and smooth (figs. 15 and 16), 47—93 µm diameter, average 62.6±6.4 µm. Their mode of formation is uncertain.

Table 2. Dimensions (in µm) of primary conidia, secondary conidia and resting spores of *E. gigantea* from *T. paludosa*, based on 50 measurements per individual (secondary conidia n = 25)

	Ind. no.	Length $\bar{x}$	($\pm s_x$)	min-max	Diameter $\bar{x}$	($\pm s_x$)	min-max
Primary conidia	4	94.8	(6.9)	80—110	79.9	(7.8)	63— 93
	15	86.8	(8.1)	73—107	70.8	(8.1)	57— 87
	21	88.9	(10.8)	57—111	73.1	(10.4)	46— 97
	22	88.0	(8.3)	71—107	70.3	(7.5)	58— 87
	49	96.4	(8.5)	77—111	80.5	(8.1)	67— 94
	50	98.5	(10.3)	80—123	84.7	(9.9)	64—105
Secondary conidia	13	76.2	(6.1)	65— 88	65.7	(5.6)	57— 76
Resting spores	37				58.4	(5.2)	48— 70
	67				59.3	(5.9)	47— 77
	70				70.0	(8.0)	53— 93

Culture: The species can easily be cultured on EYM, SDAEY and on Sabouraud dextrose-agar (SDA). On EYM and SDAEY it grows very rapidly. After one week the colonies reach a diameter of 6—10 cm. Colonies completely covering EYM are rather coarsly folded and slightly darkened (fig. 19b). On SDAEY the fungus forms an abundant mycelium which completely fills the culture tube (fig. 19a). On SDA growth is a little slower. Some of the strains produce resting spores. These are distinctly larger than those from the insects (fig. 16), whereas the differences in the dimensions of the conidia are only small (tab. 3).

Distuinguishing characters: The species is characterised by the strikingly large dimensions of conidia and resting spores and by the peculiar shape of the hyphal bodies. It is therefore easily to distuinguish from the other known species with similar conidial shape (Macleod & Müller—Kögler, 1973).

The decision to assign the present species to *Entomophthora* and not to *Conidiobolus* was based primarily on the traditional criteria. The borderline between these two genera is fluid, the separation being based, traditionally, not only on morphological criteria but primarily on their relative pathogenicity for Arthropods (MACLEOD, 1963; KING, 1976a). Such a differentiation is unsatisfactory and inaccurate(REMAUDIERE et al., 1976). Also the proposal of KING (1976b) cannot be considered as a final clarification of this situation. His criteria (conidial shape, rate of growth in culture, method of resting spore formation) lead again to an arbitrary separation and to a part of a morphologically rather homogenous group (the genus *Entomophaga* of BATKO [1964]) being included in *Conidiobolus*.

Holotype: Slides containing transverse sections of an adult *Tipula paludosa*, collected on September 6, 1977, contangini conidia and developing resting spores. Deposited in the herbarium of the Institut für spezielle Botanik, Eidgenössische Technische Hochschule, Zürich, Switzerland (ZT).

Table 3. Dimensions (in µm) of primary conidia and resting spores of *E. gigantea* from cultures, based on 50 measurements per cultre

	Cult. no.	Length $\bar{x}$	($\pm s_x$)	min-max	Diameter $\bar{x}$	($\pm s_x$)	min-max
Primary conidia	36	86.3		68—103	74.4		58— 92
	40	100.8		82—110	88.3		67—104
	66	100.1	(12.3)	77—133	88.8	(12.7)	69—127
	67	88.5	(8.8)	70—110	76.7	(9.2)	57— 96
	74	98.4	(12.2)	81—137	86.7	(13.2)	69—131
	75	93.8	(10.0)	74—119	83.2	(10.3)	65—108
Resting spores	36				86.8		69—103
	67				81.9	(9.7)	61—100
	75				79.6	(9.7)	58— 93

Type host: *Tipula paludosa* MEIG. (Diptera: Tipulidae).

Type locality: Switzerland, Zürich—Reckenholz.

Cultures: Deposited at CMI and CBS.

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

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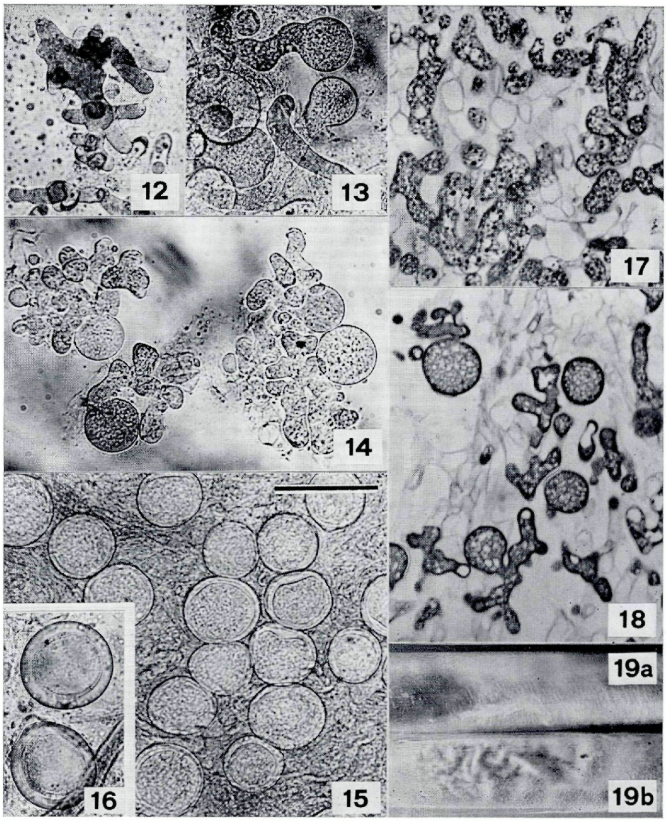

Entomophthora gigantea (figs. 12—19). 12. Single, typical hyphal body. 13. Two resting spores developing from hyphae-like hyphal bodies. 14. Four resting spores developing from the typical, amoeboid hyphal bodies. 15. Young resting spores from an insect. 16. Mature resting spores from a culture. 17. Histological section through hyphal bodies. 18. Histological section showing developing resting spores. 19. Nine-day old cultures on SDAEY (a) and on EYM (b). Bar in fig. 15 represents 100 μm — figs. 12—18, same magnification)

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