Two new species of Entomophthoraceae (Zygomycetes, Entomophthorales) linking the genera *Entomophaga* and *Eryniopsis*

S. Keller[1] & J. Eilenberg[2]

[1]Federal Research Station for Agronomy, Reckenholzstr. 191, CH-8046 Zürich, Switzerland

[2]The Royal Veterinary and Agricultural University, Department of Ecology and Molecular Biology, Bülowsvej 13, DK 1870 Frederiksberg C, Copenhagen, Denmark

Keller, S. & Eilenberg, J. (1993). Two new species of Entomophthoraceae (Zygomycetes, Entomophthorales) linking the genera *Entomophaga* and *Eryniopsis*. – Sydowia 45 (2): 264–274.

Two new species of the genus *Eryniopsis* from nematoceran Diptera are described; *E. ptychopterae* from *Ptychoptera contaminata* and *E. transitans* from *Limonia tripunctata*. Both produce primary conidia and two types of secondary conidia. The primary conidia of *E. ptychopterae* are 36–39 x 23–26 µm and those of *E. transitans* 32–43 x 22–29 µm. The two species are very similar but differ mainly in the shape of the conidia and number of nuclei they contain. Both species closely resemble members of the *Entomophaga grilly* group and probably form the missing link between *Eryniopsis* and *Entomophaga*.

Keywords: Insect pathogenic fungi, taxonomy, Diptera, Limoniidae, Ptychopteridae.

The Entomophthoraceae consists of mostly insect pathogenic fungi whose taxonomy has not been fully resolved. One controversial genus is *Eryniopsis* which is characterized by unitunicate, plurinucleate and elongate primary conidia usually produced on unbranched conidiophores and discharged by papillar eversion (Humber, 1984). Two types of secondary conidia are produced, types Ia and Ib or Ia and II (Ben Ze'ev & Kenneth, 1982).

Rhizoids may be present or absent. Most characters of the primary conidia resemble those of the genus *Entomophaga* Batko as defined by Keller (1987) [genera *Entomophaga* Batko emend. Humber (1989) and *Batkoa* Humber (1989)], while the types of the secondary conidia resemble those of the genera with uninucleate conidia, *Erynia* (Fres) Rem. & Henn. and *Zoophthora* (Batko) Rem. & Henn. (Keller, 1991a). This paper describes two new species of *Eryniopsis* confirming the intermediate position of this genus but linking it more closely to the genus *Entomophaga*, especially to the *E. grylli*–group.

The systematics of the Entomophthoraceae is not yet clearly established. We therefore follow the description of the genera given by Keller (1991b), which does not consider the split within the genera *Entomophaga* and *Erynia* proposed by Humber (1989).

Material and methods

Collection and preparation of the material

Infected *Limonia tripunctata* F. (Diptera, Limoniidae) were placed on the surface of water in small Petri dishes. The projected conidia were collected on a slide placed about 2 mm above the cadaver. The cadavers usually remained on the water surface for approximately 8 hours and were then transferred to 70% (v/v) ethanol and stored for microscopical examination. Conidia projected onto the water surface were picked up with a slide 8–14 hours after the cadavers were placed on the water surface. A certain percentage of these conidia had produced secondary or even tertiary conidia. Numerous living insects were killed and stored in ethanol.

Infected *Ptychoptera contaminata* (L.). (Diptera, Ptychopteridae) were collected either as fungus–killed cadavers, or alive. Living individuals were incubated for a few days and any dying of fungus retained as fresh specimens. Fungus–killed specimens were placed in humid chambers and conidia projected onto glass slides. After a few hours projection, some slides were then incubated for another 8 hours to observe the formation of secondary conidia, or incubated even longer to observe the formation of conidia of successive orders. After projection, cadavers were stored in 70% (v/v) ethanol until preparation took place.

Stains

Conidia and cadavers were mounted in lactophenol-cotton-blue (LPCB) (0.001–0.1%), in 1% aceto-orceine or in lactophenol-aceto-orceine according to Keller (1987) (LPAO)

Cultivation

The "GLEN"–medium (Eilenberg & al., 1992) was used to isolate and maintain the fungus from *P. contaminata*.

Counts and measurements

Unless stated otherwise, all counts and measurements were based on 50 objects per individual host, designated as 1 series. For each fungus species and structure usually several series were examined to

assess variability. The number of the 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). Single measurements of fungal structures are rounded to 1 µm, those of nuclear diameters to 0. 5 µm.

Results

Eryniopsis transitans Keller, sp. nov. – Pl. 1, Figs. 1–11.

Conidia primaria (26–)32–43(–48) x (18–)22–29(–35) µm, pyriformia vel ellipsoidea, unitunicata, 15 (7–24) nucleis diametro 4–5 µm. Conidia secundaria primariis similia (27–)30(–33) x (19–)23(–28) µm subfusiformia vel subellipsoidea, 54–77 x 13–23 µm, hyphae evolutae, 55–70 µm longae. Corpora hyphalia sphaerica aut irregularia. Conidiophora simplicia. Cystidae, rhizoidea et sporae perdurantes ignota. In Limonia tripunctata F. (Diptera, Limoniidae), hospite typico. Helvetia. Holotypus ZT, cotypi K et BPI.

Host. – Diptera, Limoniidae : *Limonia tripunctata* F. (type species).

Symptoms. – Infected, adult insects fixed to the underside of leaves.

Rhizoids absent. – Hyphal bodies more or less regularly spherical to irregular, containing 15 (11–24) nuclei with a diameter of 5.6 (5–6.5) µm (1 series), nuclei clearly staining with LPAO (fig. 5). Hyphal bodies germinate with single germ tube. – Conidiophores simple with 15–16 (12–25) nuclei with a diameter of 4.6 (4–5.5) µm (1 series; fig. 6). – Primary conidia 32.1–42.9 x 21.7–28.9 µm (26–48 x 18–35 µm), L / D = 1.34–1.49 (3 series); pyriform to ellipsoid, papilla broad, flat to slightly rounded; 15 (7–24) nuclei with a diameter of 4.5 (4–5) µm (1 series) usually with a single, prominent vacuole (figs. 7–8). – Secondary conidia like primary, 29.7 x 23.3 µm (27–33 x 19–28 µm), L / D = 1,28 (1 series, n = 27), often with very fine apical point and single prominent vacuole; on short, thick conidiospore (fig. 9); or elongate, slightly irregularly fusiform to ellipsoidal, 68.8 x 19.6 µm (54–77 x 13–23 µm), L / D = 3.52 (1 series), on tapering thick conidiophore with a length of 55–70 µm, a basal and an apical diameter of 13–18 µm and 4–5 µm respectively (1 series, n = 8) (fig. 10). – Secondary conidiophores emerge laterally from primary conidia. – Cystidia and resting spores not observed.

Distribution. – Switzerland, Katzensee ZH.

Etymology of specific epithet. – *Transitans* suggests that the species possesses characters of the genera *Entomophaga* and *Eryniopsis* and is considered a link between them.

The species was collected in late May / early June in a pure stand of stinging nettles (*Urtica dioica* L.) interspersed with trees, mainly alder (*Alnus* sp.). Although *L. tripunctata* was frequent, only a few infected specimen were found and these were attached to the underside of nettle leaves towards the top of the plants.

The species is closely related with the *Entomophaga grylli*-group, especially with *E. tipulae*, but there is also a close relationship with *Eryniopsis ptychopterae*. It differs from both by the peculiar secondary conidia, by the different host species and by the number of nuclei per conidium. It can be separated from *Entomophaga limoniae*, which attacks the same host species, by the shape and dimensions of primary conidia and by the number of nuclei per conidium.

Material from living insects suggests the following vegetative growth: multiplication of the fungus in the host body takes place by more or less spherical protoplasts. Their nuclei do not stain in LPAO. At a later stage a single large nucleus with loose chromatin structure becomes visible (Fig. 1). The protoplasts then develop to spherical or subspherical hyphal bodies containing 2 nuclei with loose chromatin (Fig. 2). The nuclei continue to divide to form 4–nucleate and 8–nucleate hyphal bodies (Figs. 3–4). During this process the hyphal bodies slightly increase in size. There were indications that the 4–nucleate hyphal bodies produce bi–nucleate hyphal bodies. A typical, fully developed hyphal body is spherical, subspherical, irregularly subspherical or elongate and contains 15–16 nuclei (Fig. 5).

Eryniopsis ptychopterae Keller & Eilenberg, sp. nov. – Pl. 2, Figs. 1–9.

Conidia primaria (28–)36–39(–51) x (18–)23–26(–33) µm, pyriformia, unitunicata, 20–22 (16–28) nucleis. Conidia secundaria primariis similia 27–41 x 17–30 µm, subcylindriformia vel subfusiformia 38–64 x 12–22 µm. Corpora hyphalia sphaerica vel subsphaerica, 18–26 (11–42) nucleis diametro 4. 5 (3. 5–5. 5) µm. Conidiophora simplicia. Rhizoidea, cystidae et sporae perdurantes ignota. In Ptychoptera maculata M. (Diptera, Ptychopteridae), hospite typico. Denmark, Holotypus ZT , cotypi K et BPI.

Host. – Diptera, Ptychopteridae: *Ptychoptera contaminata* M.

Symptoms. – Dead adult *P. contaminata* fixed their legs to plants in and at the edge of a creek between a few and about 50 centimeters above the water level, head upwards (Fig. 1).

Rhizoids absent. – Hyphal bodies spherical, subspherical or irregularly subspherical, with 18–26 nuclei (11– 42) with a diameter of 4.5–4.6 (3.5–5.5) µm (3 series), germinating with a single germ tube. – Conidiophores simple, 21–22 (15–29) nuclei with a dia-

meter of 4.7 (4–5.5) µm (2 series; fig. 2). – Primary conidia 35.8–39.3 x 22.9–25.8 µm (28–51 x 18–33 µm), L / D = 1.43–1.69 (7 series), pyriform, largest diameter more or less in the middle, usually with 1(–2) prominent vacuole(s) and 20–22 (16–28) nuclei, apex rounded, papilla prominent and rounded (figs. 3–4). – Secondary conidia like primary, 35.1–35.6 x 23.5–25.8 (27–41 x 17–30) µm, L / D = 1.36–1.43 (2 series) produced on very short, thick lateral conidiophores sometimes emerging directly lateral from primary conidium (fig. 6); or subcylindrical to ellipsoidal, 48.8–55.9 x 16.4–17.4 µm (38–64 x 12–21 µm), L / D = 2.89–3.39 (4 series), straight or slightly bent, papilla conical, pointed or indistinct produced on thick, elongate conidiophore with a length of about 22–54 µm, a basal and an apical diameter of 4.5–10 µm and 3 µm respectively (1 series, n = 10; Figs. 5, 7). – Tertiary conidia like primary and secondary, 36.0 x 23.7 µm (34.5–37.5 x 22.5–24.8), L / D = 1.50 (1 series), produced by both types of secondary spores on thick, lateral conidiophores emerging directly from secondary conidium (Fig. 8), or subcylindrical to ellipsoidal, produced on thick elongate conidiophore emerging from subcylindrical secondary spore. – Cystidia and resting spores not observed.

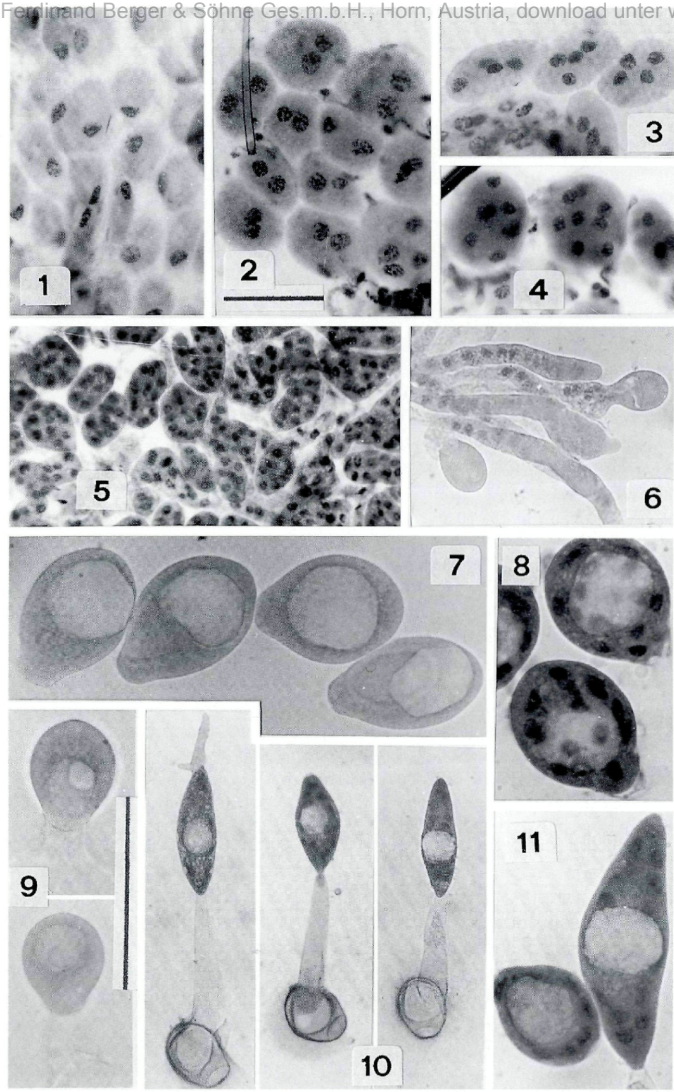
Cultural characters. – The species was isolated in liquid medium ("GLEN") by allowing primary conidia to drop into the medium (Eilenberg & al., 1992). The hyphal bodies contained 28 (16–53) nuclei with a diameter of 4.6 (3.5–6.0) µm. The primary conidia measured 56.7–61.4 x 36.6–38.6 µm (45–79 x 31–51 µm), L / D = 1.55–1.59 (2 series).

Distribution. – Denmark, Kattinge (near Roskilde, Zealand).

Etymology of specific epithet. – Suggesting the genus of the host from which the fungus was first collected.

The species was collected in the August of several years between 1983 and 1992, occasionally in large numbers. It is characterised by *Entomophaga grylli*–like primary conidia and the two types of secondary conidia which resemble *Eryniopsis caroliniana* (Keller, 1978; 1991a), from which it can, however, easily be separated by the shape of the conidia and the number of nuclei. It closely resembles *Eryniopsis transitans*. Details for the separation are given under that description.

Observations on the formation of secondary and successive order conidia could only document the production of type 1a tertiary conidia from type 1a secondary conidia, whereas a type 1b tertiary conidium was observed being produced from a type 1b secondary



Pl. 1. – 1–11: *Eryniopsis transitans*. –1–4. Vegetative growth. – 1. Mononucleate spherical to subspherical protoplasts / hyphal bodies. – 2. Hyphal bodies with 2 or 4 nuclei with loose chromatin structure. – 3. 4–nucleate hyphal bodies. – 4. One 4–nucleate and two 8–nucleate hyphal bodies, nuclei with dense chromatin structure. – 5. Fully developed hyphal bodies with nuclei. – 6. Conidiophores and formation of primary conidia. – 7. Primary conidia with single prominent vacuole. – 8. Primary conidia with nuclei. – 9. Secondary conidia of type Ia with slightly visible apical point. – 10. Secondary conidia, one with apical germ tube, and the remainings of primary conidia and conidiophores. – 11. Secondary conidia of type Ia and Ib with nuclei. – 1–6, 8, 10–11: LPAO, 7, 9: LPCB. – Bar in figs. 2 and 10: 50 µm; 1–6, 10; 7–9, 11: same magnifications.

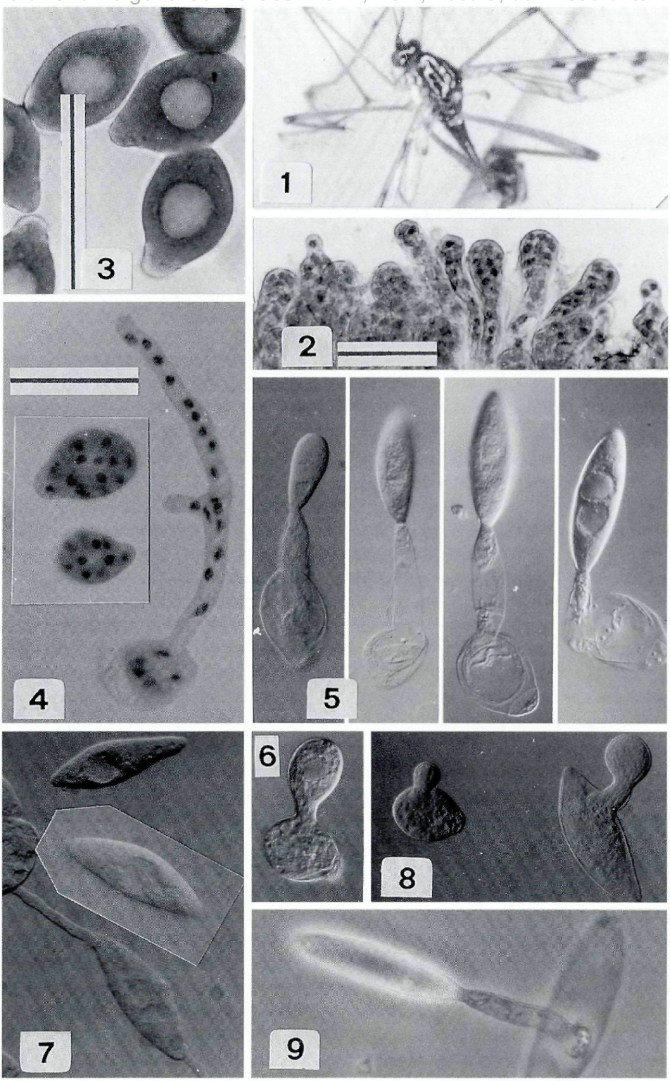
conidium (Fig. 9). Only type 1a quarternary conidia were found. Occasionally a sequence of up to four type 1a conidia was formed without being discharged. Conidia of all types and degrees of formation may germinate to produce mycelium. Single germ tubes emerged at one end or both ends of the conidia. From primary conidia or type 1a secondary conidia or tertiary conidia the germ tubes emerged mostly laterally, while these from type 1b secondary conidia emerged axially.

Discussion

The genus *Eryniopsis* was established to include species with characters intermediate between *Entomophaga* and *Erynia/Zoophthora* (Humber, 1984). Until now it consisted of *E. caroliniana*, *E. lampyridarum* and *E. longispora*. Their resemblance to *Entomophaga* was expressed by the unitunicate, plurinucleate conidia, while that with *Erynia/Zoophthora* was expressed in part by the two types of secondary conidia, partly by the branched conidiophores and by the shape of the primary conidia.

The two species described here closely resemble those of the genus *Entomophaga*. On the basis of primary spore morphology, these isolates could easily be mistaken as members of the *Entomophaga grylli* group, especially *E. tipulae* which occurs on related hosts (Tab. 1). Only the presence of the two types of secondary conidia makes it clear that they belong to *Eryniopsis* which demonstrates the taxonomic importance of secondary conidia characteristics. Because of this close relationship to *Entomophaga*, we consider these two new species as a link between the two genera. *Eryniopsis* shows a larger genetic variability than *Entomophaga*, *Erynia* and *Zoophthora*. With regard to evolutionary aspects we propose that *Eryniopsis* is the ancestor of both *Entomophaga* and *Erynia/Zoopthora* although the link with the latter has still to be established.

Within the genus *Eryniopsis*, *E. ptychopterae* and *E. transitans* are characterised by the pyriform shape of the primary conidia and the distinctly larger number of nuclei and like *E. caroliniana* and *E. lampyridarum* they produce two types of secondary conidia. The first resembles the primary ones (type Ia) while the other is morphologically different. Type Ib conidia of *Eryniopsis* are not rounded as those of the genus *Erynia*. Its shape in all four species is subcylindrical, ellipsoidal or subfusiform. Except for *E. lampyridarum* both types of secondary conidia are produced on thick conidiophores. While those of type Ia secondary conidia are short (except for occasionally longer ones in *E. ptychopterae*), those of type Ib are much longer. The longer they are the more they taper. This fact probably also influences the mode of detachment. In *E. caroliniana* both types of secondary conidia are actively discharged which is also strongly indicated for *E.*



Pl. 2. – 1–9: *Eryniopsis ptychopterae*. – 1. Diseased *Ptychoptera contaminata* hanging on leaf blade (ca. 5 x nat. size) . – 2. Tips of conidiophores with nuclei immediately before formation of conidia starts. – 3. Primary conidia. – 4. Primary conidia, one germinated, with nuclei. – 5. Formation of type Ib secondary conidia. – 6. Formation of type Ia secondary conidia. – 7. Detached type Ib secondary conidia, one with a germ tube at both ends. – 8. Type Ia and type Ib secondary conidia germinating to form type Ia tertiary conidia. – 9. Type Ib secondary conidia with fully developed type Ib tertiary conidia. – 2, 4: LPAO; 3, 5–9: LPCB. – Bar in figs. 2, 3 and 4: 50 µm; 4–9: same magnifcations.

Tab. 1. – Conidial dimensions and nuclear numbers in all described species of *Eryniopsis* and in closely related species of *Entomophaga*.

	caroliniana [1]	*lampyridarum* [2]	*longispora* [3]	*ptychoptera*	*transitans*	*tipulae* [4]	*grylli*
Dimensions (in µm)							
prim. conidia	28–43 x 13–15	36.5 ± 1.1 x 17.1 ±2.6	40–75 x 7–9	36–39 x 23–26	32–43 x 22–29	35–42 x 27–30	36–41 x 26–31
L / D	1.9–2.8	2.1		1.4–1.7	1.3–1.5	1.3–1.5	1.3–1.4
second. conidia							
type I a	21–29 x 12–18	present	?	35–36 x 23–26	30 x 23	35 x 26	28–30 x 22–24
L/D	1.6–1.8			1.4	1.3	1.3	1.3
type I b	35–43 x 9–10	absent	?	49–56 x 16–17	69 x 20	absent	absent
L / D	3.7–4.5			2.9–3.4	3.5		
type II	absent	37.7 ± 1.1 x 15.3 ±0.2	?	absent	absent	absent	absent
L / D		2.5					
Number of nuclei							
hyphal bodies	3–10		4–10	18–26 (11–42)	15–16 (12–25)	25–28 (17–37)	26 (13–33)
prim. conidia	4–10	4–6 (?)	4–10	20–22 (16–28)	15 (7–24)		25–28 (11–33)

[1]) Data from Keller (1978. 1987, 1991a)
[2]) Data from Carner (1980) and Humber (1984)
[3]) Data from Balazy (1982)
[4]) Data from Keller (unpubl.)

ptychopterae. For *E. transitans* the mode of detachment of the type Ib secondary conidia is unknown. The narrow tip of the secondary conidiophore, however, suggests passive detachment, seen also in *E. lampyridarum*, which has capillary secondary conidiophores and passively detaching type II secondary conidia (Carner, 1980). *E. transitans* may therefore represent another transition, that from actively to passively discharged secondary conidia or from type Ib (or yet to be defined type Ic) to type II secondary conidia.

Type Ib secondary conidia are the dominant type of secondary conidia of *E. caroliniana* (Keller, 1977). In contrast to this finding this type was rare in both new species and found only on a few slides. It seems to be produced only under particular conditions.

From the point of view of the host it is noteworthy that three of the now five known *Eryniopsis* species attack hosts of closely related families of Nematocera living in similar habitats. Further more members of these insect families or even the same species are hosts of entomophthoralean fungi of the genera *Entomophaga, Erynia* and *Zoophthora*. These insects could be of particular importance for the evolution of the Entomophthoraceae.

Acknowledgments

The authors are greatly indepted to Drs. T. M. Butt and Judith Pell for critically reviewing the manuscript, O. Petrini for helping with the latin diagnosis, and W. Geiger and W. Michelsen for identifying the hosts.

References

Balazy, S. (1982). New species of Entomophthoraceae from the Wielkopolski National Park. – Bull. Acad. Pol. Sci., Sér. sc. biol. cl. II 29: 221–226.

Ben Ze'ev, I. & R. G. Kenneth (1982). Features-criteria of taxonomic value in the Entomophthorales : I. A revision of the Batkoan classification. – Mycotaxon 14 : 393 : 455.

Carner, G. R. (1980). *Entomophthora lampyridarum*, a fungal pathogen of the soldier beetle, *Chauliognathus pennsylvanicus*. – J. Invertebr. Pathol. 36: 394–398.

Eilenberg, J., J. Bresciani & N. Wilding (1992). Isolation *in vitro* of *Strongwellsea castrans*, a pathogen of adult cabbage root flies, *Delia radicum*. – Entomophaga 37: 65–77.

Humber, R. A. (1984). *Eryniopsis*, a new genus of the Entomophthoraceae (Entomophthorales). – Mycotaxon 21: 257–264.

—— (1989). Synopsis of a revised classification for the Entomophthorales (Zygomycotina). – Mycotaxon 34 : 441–460.

Keller, S. (1977). Über zwei Entomophthorosen von *Tipula paludosa* Meig. – Mitt. Schweiz. Ent. Ges. 50: 277–284.

—— (1978). *Entomophthora gigantea* sp. nov. and *E. caroliniana* (Thaxter) comb. nov., two pathogens of *Tipula paludosa* MEIG. – Sydowia 31: 87–93.

—— (1987). Arthropod-pathogenic Entomophthorales of Switzerland. I. *Conidiobolus, Entomophaga* and *Entomophthora*. – Sydowia 40: 122–167.
—— (1991a). Arthropod-pathogenic Entomophthorales of Switzerland II. *Erynia, Eryniopsis, Neozygites, Zoophthora* and *Tarichium*. – Sydowia 43: 39–122.
—— (1991b). Entomophthorales: A key for the indentification of the arthropod-pathogenic genera and their characterisation. – IOBC / WPRS Bull. 1991 / XIV / 7: 124–129.

(Manuscript accepted 15th April 1993)