The genus *Neozygites* (Zygomycetes, Entomophthorales) with special reference to species found in tropical regions

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Keller, S. (1997). The genus *Neozygites* (Zygomycetes, Entomophthorales) with special reference to species found in tropical regions. – Sydowia 49(2): 118–146.

Several collections of mites (Acari: Tetranychidae, Phytoseiidae), mealybugs (Homoptera: Pseudococcidae) and aphids (Homoptera: Aphididae, Lachnidae) infected with Entomophthorales mainly from West Africa, South America and the Philippines were examined. All fungi found on Tetranychidae including the green cassava mite, *Mononychellus tanajoa*, were assigned to *N. floridana*. Species found on the phytoseiid *Euseius citrifolius* were identified as *N. acaricida* comb. nov. and *N.* cf. *acaridis*, respectively. All material from mealybugs (*Rastrococcus invadens* and *Coccidohystrix insolita*) was identified as *N. fumosa*. An emended description of this species is given. The fungi from *Aphis craccivora* and *A. fabae* were identified as *N. fresenii*. A new species, *N. cinarae* attacking the lachnid *Cinara pilicornis*, is described. The life-cycles of *N. floridana* and *N. fresenii* are described and compared. A key to all known species and a short characterisation is provided.

Keywords: *Neozygites*, taxonomy, life cycle, entomopathogens, Entomophthorales, Acari, Homoptera, Aphidae, Pseudococcidae, tropics.

The genus *Neozygites* Witlaczil (1885) includes species with spherical or rod-shaped hyphal bodies; unbranched conidiophores; spherical to obovate primary conidia with 3–8 nuclei; presence of capilliconidia with typically bent capillary tubes; spherical or ellipsoidal, binucleate, dark brown to black zygospores, rarely azygospores; cystidia always absent, rhizoids normally absent.

The nuclear structure and the nuclear behaviour during mitosis of *Neozygites* differs from the other Entomophthorales (Butt & Heath, 1988; Butt & Humber, 1989). Therefore the genus was placed in a distinct family, Neozygitaceae Ben-Ze'ev & Kenneth (Ben-Ze'ev & al., 1987).

Ben-Ze'ev & al. (1987) proposed a new genus *Thaxterosporium* to include a single species, *N. turbinata* (Kenneth & Ben-Ze'ev) Remaudière & Keller (1980). Due to reasons discussed by Keller (1991) and Villacarlos & Wilding (1994) this genus was synonymised with *Neozygites* (Keller, 1991).

Neozygites consists of a homogenous group of fungi attacking small pterygote insects (Homoptera, Thysanoptera) and mites. Recently, another species was discovered attacking the apterygote springtail *Sminthurus viridis* L. (Keller & Steenberg, 1997).

Members of *Neozygites* have recently been recognised as important natural control agents of the cassava green mite, *Mononychellus tanajoa* (Bondar), and as potential mycoinsecticides to control mites and thrips. It was mainly the work in the cassava ecosystem in Brazil and in West Africa which resulted in several collections of *Neozygites* from phytophagous and predatory mites and from mealybugs. The species attacking *M. tanajoa* has been referred to as *Neozygites* sp. (e.g. Delalibera & al., 1992) or more recently as *N.* cf. *floridana* (e.g. Oduor, 1995, Yaninek & al., 1996).

Several collections originating from different species of mites, mealybugs and aphids were sent to the author for identification, the results of which are presented here. Data on the biology of these species are compared and discussed with respect to their systematic and taxonomic relevance and a key to all described species is provided.

Material and methods

The fungal material examined is listed in Tab. 1. It was sent either prepared and mounted on slides, stored in ethanol, as exsiccata, or in a living air-dried state allowing the induction of sporulation.

The fungal material was mounted on lactophenol-cotton blue (LPCB) or in lactophenol-aceto-orcein (LPAO) as described by Keller (1987). A sample of mites stored in ethanol was stained with 4,6-diamidino-2-phenylindol (DAPI) to confirm the nuclear counts (Butt & Humber, 1989; Butt & al. 1989). All measurements and counts were based, if not otherwise stated, on 50 objects per individual host, designated as one series. From each fungus species and origin, usually more than one series was studied 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).

### Taxonomy

Neozygites acaricida

The GJM/unid collection consisted of mites (*Euseius citrifolius* Denmark & Muma) mounted for acarological studies including removal of cytoplasmic content of both host and pathogen. It contained four mites with spherical to subspherical, hyaline, thick-walled, resting spores with diameters varying between 11.5 and 13 μm. They are slightly larger than those given by Milner (pers. comm.).

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Tab. 1. – Material of *Neozygites* examined.

Species	Origin of the material	Collection (designation)	year
cf. *acaricida*	G. J. de Moraes, Brazil, *Euseius citrifolius*	GJM/Ec2	1995–96
cf. *acaridis*	G. J. de Moraes, Brazil, *E. citrifolius*	GJM/Ec1	1995
floridana	M. H. Muma (paratype material), Florida, USA. *Eutetranychus banksi*	MHM/Eb	1963–64
floridana	J. Weiser (paratype material), Florida, USA. *E. banksi*	JW/Eb	1963–64
floridana	D. R. Smitley, Carolina, USA. *Tetranychus urticae*	DRS/Tu	1982
floridana	S. Keller. Switzerland, *T. urticae*	SK/Tu	1983–86
cf. *floridana*	I. Delalibera. Brazil, *Mononychellus tanajoa*	ID/Mt	1994
cf. *floridana*	J. S. Yaninek. Benin, *M. tanajoa*	JSY/Mt	1994–96
cf. *floridana*	J. S. Yaninek. Benin, *T. urticae*	JSY/Tu	1996
cf. *floridana*	L. Smith, Colombia, *M. tanajoa*	LS/Mt	1996
cf. *floridana*	L. Smith, Colombia, *T. urticae*	LS/Tu	1996
cf. *floridana*	S. L. Elliot, Brazil, *M. tanajoa*	SLE/Mt	1995
cf. *floridana*	S. L. Elliot, Brazil, *T. urticae*	SLE/Tu	1995
fresenii	S. Keller, Switzerland, *Aphis* spp.	SK/Aspp	1985
cf. *fresenii*	S. Keller, Benin, *A. craccivora*	SK/Ac	1996
cf. *fresenii*	L. T. Villacarlos, Philippines, *A. craccivora, A. fabae*	LTV/Ac	1995
cf. *fresenii*	P. Wyss, Egypt, *Aphis gossypii*	PW/Ag	1996
fumosa	A. T. Speare (holotype), Florida, USA Pseudococcus citri; nrs. 737879 and 737880	BPI	1923
fumosa	B. Le Rü, Congo-Brazzaville, *Phenacoccus manihoti*	BLR/Pm	1986
cf. *fumosa*	C. J. Lomer, Benin, *Rastrococcus invadens*	CJL/Ri	1996
cf. *fumosa*	L. T. Villacarlos, Philippines, *Coccidohystrix insolita*	LTV/Ci	1996
cf. *fumosa*	L. T. Villacarlos, Philippines, mealybug	LTV/unid	1996
tetranychi	J. Weiser, Czechoslovakia, paratype, *Tetranychus* spp.	JW/Tspp	1966
unidentified	G. J. de Moraes, Brazil, *E. citrifolius*	GJM/unid	1995

The same collection contained several other mites with spherical, hyaline, thin-walled structures with an average diameter of 10.0 μm (8–11 μm) (1 series) considered as hyphal bodies (Plate 1, Figs. 8–9). Some of the hyphal bodies germinated to produce a single conidium of similar shape on a short germ tube. Undetached conidia measured 10.6×7.3 μm ($10–12 \times 6–8$ μm) (1 series, n = 10). The GJM/Ec2 col-

Plate 1. – Figs. 1–5: *N. floridana* from *Mononychellus tanajoa*. – 1. Stouted conidiophores. – 2. Under host cuticle stunted conidiophores, some have penetrated the cuticle and form primary conidia. – 3. Primary conidia. – 4. Capilliconidia. Inlet: Detached capilliconidia with haptor. – 5. Detached capilliconidia attached to mite leg. – 6–7: Resting spores of *N.* cf. *acaridis* from *Euseius citrifolius*, some of them with clearly visible ornamentation (6). – 8–9: Unidentified structures in *E. citrifolius*. – 8. Mite filled with spherical bodies. – 9. Spherical structures. – Bars in Figs. 5 and 8 = 50 μm, Figs. 1–7, 9 same magnification.
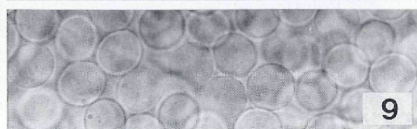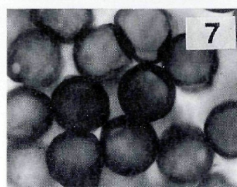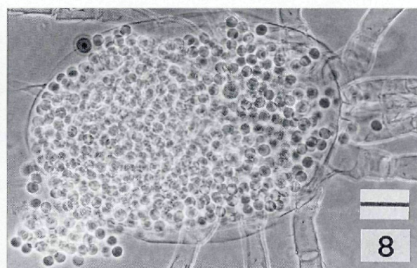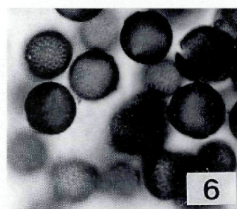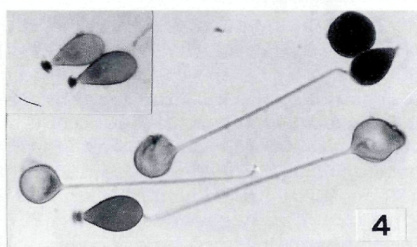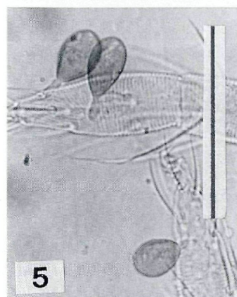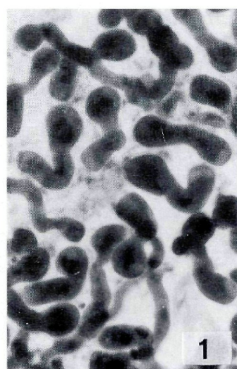
lection consisted of mites stored in ethanol infected with the same fungus. Hyphal bodies measured 9.1–10.1 μm (8–12 μm) (3 series) and contained 3 or more frequently 4 nuclei. The nuclei stained indistinctly in LPAO but very clearly in DAPI. Undetached primary conidia were ovoid and measured 11.3×7.1 μm ($11–12 \times 6–7.5$ μm) (1series, n = 8).

Measurements for hyphal bodies and conidia match the description of *Empusa acaricida* Petch (1940) from *Hylotydeus destructor* (Tucker) and it is concluded that the two fungi are identical. The structures closely resemble those recently described for *N. sminthuri* (Keller & Steenberg, 1997) and, therefore, attributed to *Neozygites*. This is in agreement with Milner who collected *E. acaricida* on two mite species in Australia, including the original host, and considered it another species of *Neozygites* lacking capilliconidia (Milner, 1985 and pers. comm.).

Neozygites cf. *acaridis*

The collection GJM/Ec1 consisted of several mites mounted for acarological studies including removal of the cytoplasmatic content of both host and pathogen. Some mites contained hyaline, spherical to slightly subspherical structures considered as hyphal bodies. They measured $12.6–12.9$ μm $\times 12.2–12.5$ μm ($11–14 \times 10–13$ μm) (2 series). One mite contained spherical, dark brown resting spores with a diameter of 15.9 μm (14–17 μm) (1 series) including the episporium and 14.1 μm (13–16 μm) without episporium. Another mite contained the same type of resting spore measuring 15.7 μm (14–17 μm) (1 series) together with spherical hyphal bodies with a diameter of 11.0 μm (10–12 μm) (1 series). The resting spores in both mites had a distinct hylum and the episporium had finely pointed ornamentation (Plate 1, Figs. 6–7).

The dimensions of the resting spores match the description of *N. acaridis* given by Milner (1985) but the hyphal bodies are distinctly smaller. Although hyphal bodies producing conidia are larger than those producing zygospores, it cannot be concluded with certainty that this fungus is identical with *N. acaridis*.

Neozygites floridana

No holotype material was available, but material identified by the authors of the original description (collections MHM/Eb and JW/Eb, Tab. 1), which must be considered as paratype material. It consisted of slides with mounted mites. In most slides the mounting fluid had partly evaporated and partly condensed. It was not possible to remount the material and no new measurements could be

Tab. 2. – Data of fungal structures on *N. floridana* and fungi attributed to this species. Measurements in μm; n = number of series of 50 measurements each. PC = primary conidia, SCII = secondary conidia type II (capilliconidia), Cap = Length of capillary tube, RS = resting spores.

Fungal structure	Collection	n	Length L		Diameter D		L/D
			x	min–max	x	min–max	
PC	orig. descr.[1]		15	13–18	12	11–13	1.25
	MHM/Eb	2	12.5–12.7	9–16	10.0–10.3	8–14	1.23–1.25
	JW/Eb	1	13.7	11–16	9.5	8–12	1.44
	SK/Tu	5	13.9–14.8	12–18	11.4–12.7	10–16	1.14–1.25
	ID/Mt	6	13.7–15.3	12–18	11.6–12.4	10–16	1.17–1.20
	JSY/Mt	6[2]	13.3–14.9	12–16	10.4–12.4	10–13	1.15–1.28
	JSY/Tu	3[3]	13.8–14.1	12–16	11.3–11.8	10–13	1.18–1.22
	LS/Mt	2	13.9–14.2	12–16	12.0–12.1	11–13	1.16–1.18
	LS/Tu	2	13.2–13.3	12–14	11.2–11.5	10–12	1.16–1.17
	SLE/Tu	1	14.1	13–16	12.1	11–15	1.16
SCII	orig. descr.[1]			15–20		10–12	
	MHM/Eb	1	14.5	11–17	9.8	8–12	1.48
	DRS/Tu	1	18.2	17–19	8.7	7–11	2.09
	SK/Tu	5	17.2–18.9	15–22	8.3–9.3	7–11	1.98–2.25
	ID/Mt	5	15.2–18.5	13–22	9.2–10.8	8–12	1.63–1.87
	JSY/Mt	2	15.3–16.8	13–19	8.3–8.6	7–10	1.83–1.94
	LS/Tu	1	18.5	16–21	8.7	7–10	2.12
Cap	orig. descr.[1]			50–60			
	DRS/Tu	1	58.7	42–70			
	SK/Tu	2	42.7–48.8	25–65			
	ID/Mt	4	45.0–50.0	28–69			
	JSY/Mt	2	47.0–52.8	27–77			
RS	orig. descr.[1]			22–26		20–24	
	DRS/Tu	3	19.6–22.1	18–27	14.6–16.6	13–18	1.18–1.34
	SK/Tu	8	19.3–23.1	16–25	18.4–21.1	16–24	1.05–1.13
	SLE/Mt	3	22.9–24.1	18–28	21.4–22.5	18–28	1.05–1.12

[1] Weiser & Muma (1966)

[2] including 2 series with 30 and 34 measurements

[3] including 2 series with 41 and 43 measurements

made by the author. The primary conidia especially in the JW/Eb collection looked slightly deformed giving them a narrow appearance with protruded, sometimes strangled papillae. This might be the reason for their smaller size, in particular the diameter, as compared to the other collections (Tab. 2). However, there is an overlap between them and the overall appearance of the fungus was the same as in all examined collections. In addition to the data listed in Tab. 2, more structures in the type material were measured. The

MHM/Eb collection contained 4 intact resting spores measuring 22–25 × 20–22 µm. The JW/Eb collection contained 6 capilliconidia measuring 17–18 × 8–11 µm with a L/D ratio of 1.8.

The data of the examined fungal structures of the other collections of *N. floridana* and the fungi attributed to this species are summarized in Tab. 2. In general, there is a good agreement of all collections with the original description. This is especially true for the species collected in Brazil and Benin on *M. tanajoa* (Plate 1, Figs. 1–5). Resting spores from *T. urticae* and *M. tanajoa* had the same rough surface structure (Plate 4, Figs. 4–5). Based on the morphological data the examined material is identical to *N. floridana*. According to the original description, this species has 4-nucleate conidia. Several studies showed that the number of nuclei in conidia, conidiophores or hyphal bodies varied between 3 and 5 but the 4-nucleate structures were dominant. Previously unpublished data from the SK/Tu collection demonstrate the inconsistency of nuclear numbers. In four individuals the nuclei were counted in 50 hyphal bodies each. 14%, 0%, 0% and 100% of them contained 3 nuclei; 38%, 24%, 100% and 0% contained 4 nuclei and 48%, 76%, 0% and 0% contained 5 nuclei. From 25 primary conidia 4, 9 and 12 contained 3, 4 and 5 nuclei respectively.

In addition to the data listed in Tab. 2, more structures were measured and counted in a few specimens. In the JSY/Mt collection 7 capilliconidia measuring on average 15.9 × 9.7 µm and 15 capillary tubes with a mean length of 54.3 µm (39–65 µm) were found. The hyphal bodies contained 3 (26%) or 4 (74%) nuclei (n = 19). The ID/Mt material contained a mite with mainly ruptured dark brown resting spores. Six apparently intact spores had a diameter of 23–27 µm. Nuclei in hyphal bodies or young conidiophores had a mean diameter of 2.9 µm (2.5–3.5 µm) (n = 25). In the JSY/Tu collection more than 90% of the hyphal bodies contained 4 nuclei (2 series, n = 50 and n = 25) with a mean diameter of 2.6 µm (2.5–3.0 µm) (1 series). The SLE/Mt collection contained cadavers with resting spores as well as rhizoids. This has already been reported for *N. floridana* infecting *Tetranychus urticae* (Keller & Wuest, 1983) and has hitherto not been recorded from other species of this genus

Neozygites fresenii

Primary conidia of the SK/Ac collection measured 18.2–18.9 × 15.6–15.8 µm (17–22 × 14–19 µm), L/D = 1.17–1.20 (2 series). Those of the LTV/Ac collection measured 16.3–17.4 × 14.1–14.5 µm (15–18 × 12–16 µm), L/D = 1.16–1.20 (2 series). Capilliconidia of the SK/Ac collection measured 20.8 × 12.8 µm (18–25 × 10–16 µm), L/D = 1.63 (1 series), those of the LTV/Ac collection 19.8–23.4 × 12.2–12.7 µm

(15–25 × 10–15 μm), L/D = 1.56–1.92 (2 series) and those of PW/Ag 20.7 × 12.9 μm (16–25 × 10–16 μm), L/D = 1.60 (1 series). The capillary tube in the SK/Ac and the LTV/Ac material had a mean length of 21.3 (12–46) μm and 16.9 (10–29) μm respectively (1 series each). Conidiogenous hyphal bodies in the LTV/Ac material had a mean diameter of 16.3 (15–18) μm (1 series).

These data generally match the description given for this species by Keller (1991), except the capillary tubes are shorter, and the primary conidia from the LTV/Ac material are slightly smaller, but overlap with measurements given by Silvie & Papierok (1991) for material collected in Tchad.

Neozygites fumosa

The type material of *N. fumosa* consisted of two envelopes with a limited number of diseased *Pseudococcus citri* with primary conidia only. They measured 18.6–19.2 × 11.0–11.1 μm (Tab. 3). The length matches the original description whereas the diameter is slightly larger. Since no other structures were found, additional data given by Speare (1922) could not be verified. However, the dimension given by him for the capilliconidia must be considered doubtful as they are far outside the size relation with primary conidia usual for this genus.

The measurements of the examined fungal structures of *N. fumosa* and of the fungi attributed to this species vary widely (Tab. 3). Primary conidia of CJL material matches the original description while those of BLR and LTV/unid (collected from the weed *Sida* sp.) material are slightly smaller and those of LTV/Ci material are distinctly smaller. The latter also has smaller conidiogenous hyphal bodies than the other material. BLR, CJL and LTV/unid material can be considered as identical with *N. fumosa*, whereas the LTV/Ci material is too limited to draw conclusions on identity or distinctness. Nuclei were counted in CJL/Ri material where no resting spores were present. From 3 series of protoplasts 8%, 4% and 10% were binucleate, 44%, 96% and 80% were trinucleate and 48%, 0% and 10% were quadrinucleate. From 4 series of hyphal bodies 8%, 6%, 0% and 0% were binucleate, 92%, 94%, 2% and 100% were trinucleate and 0%, 0%, 98% and 0 % were quadrinucleate. In a series of conidiophores 2% were binucleate and 98% trinucleate. More than 4 nuclei were never found.

Based on the material examined (except LTV/Ci) an amended description of *N. fumosa* is given (Plate 2, Figs. 1–10).

P r o t o p l a s t s irregularly rounded with 3 (2–4) nuclei with a diameter of 2.7–2.9 μm (2.5–3.5 μm) (2 series). – H y p h a l b o d i e s spherical to slightly subspherical. The spherical ones measure on

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Tab. 3. – Data of fungal structures of *N. fumosa* and fungi attributed to this species. Measurements in μm. n = number of series with 50 measurements each. PC = primary conidia, SCII = secondary conidia type II (capilliconidia), Cap. = length of capillary tube, RS = resting spores, HBco = conidiogenous hyphal bodies, HBsp = zygosporogenous hyphal bodies.

| Fungal structure	Collection	n	Length L		Diameter D		L/D
			x	min–max	x	min–max	
PC	orig. descr. [1]			16–20		8–10	
	BPI	3[2]	18.6–19.2	15–24	11.0–11.1	8–13	1.68–1.74
	BLR/Pm	3	16.9–17.6	16–21	10.2–11.3	8–13	1.55–1.65
	CJL/Ri	4	19.4–20.8	16–24	9.8–11.7	8–13	1.79–2.04
	LTV/Ci	1	14.8	13–16	7.9	7–9	1.87
	LTV/unid	5	17.1–18.2	15–21	8.2–8.9	7–10	1.99–2.13
SCII	orig. descr. [1]		8		4		
	CJL/Ri	4	15.6–16.3	13–19	8.5–9.1	7–11	1.74–1.89
	LTV/unid	3	13.8–14.8	11–18	6.7–7.3	5–8	1.98–2.13
Cap	BLR/Pm	1	46.2	27–75			
	CJL/Ri	2	57.7–67.2	34–86			
	LTV/unid	2[3]	74.1–85.8	45–139			
RS	orig. descr. [1]	15					
	BLR/Pm	4			17.3–19.4	15–23	
	LTV/unid	1			17.8	16–18	
HBco	BLR/Pm	1			12.0	10–13	
	CJL/Ri	5			11.2–12.8	10–16	
	LTV/Ci	1			10.1	8–12	
	LTV/unid	4			10.6–11.4	8–13	
HBsp	BLR/Pm	2			11.5–11.6	11–13	

[1] Speare (1922)
[2] including 1 series with n = 27
[3] n = 21 and 41

average 10.6–12.8 μm (12 series). The conidiogenous ones usually contain 3 or 4, sometimes 2 nuclei, with a diameter of 2.4–2.7 μm (2–3.5 μm) (4 series). They germinate to form a single, unbranched conidiophore. – P r i m a r y   c o n i d i a on average 16.9–20.8 × 8.2–11.7 μm (15–24 × 7–13 μm), L/D = 1.55–2.13 (15 series), usually spindle shaped with the largest diameter in the apical half, papilla distinct, narrow;

Plate 2. – Figs. 1–8: *N. fumosa* from *Rastrococcus invadens*. – 1. Protoplasts. – 2. Protoplasts turning to hyphal bodies. – 3. Hyphal bodies with nuclei. – 4. Details of trinucleate hyphal bodies. – 5–6. Primary conidia, two of them are atypically rounded and have broad papilla (arrowhead). – 7. Primary conidia and capilliconidium. – 8. Primary conidia, capillary tube and detached capilliconidium. – 9–10: *N. fumosa* from *Phenacoccus manihoti*. – 9. Conjugating hyphal bodies with developing zygospore. – 10. Mature zygospores with zwo nuclei. – Bar in Fig. 7 = 50 μm. 1–3, 5–10 same magnification; bar in Fig. 4 = 10 μm.
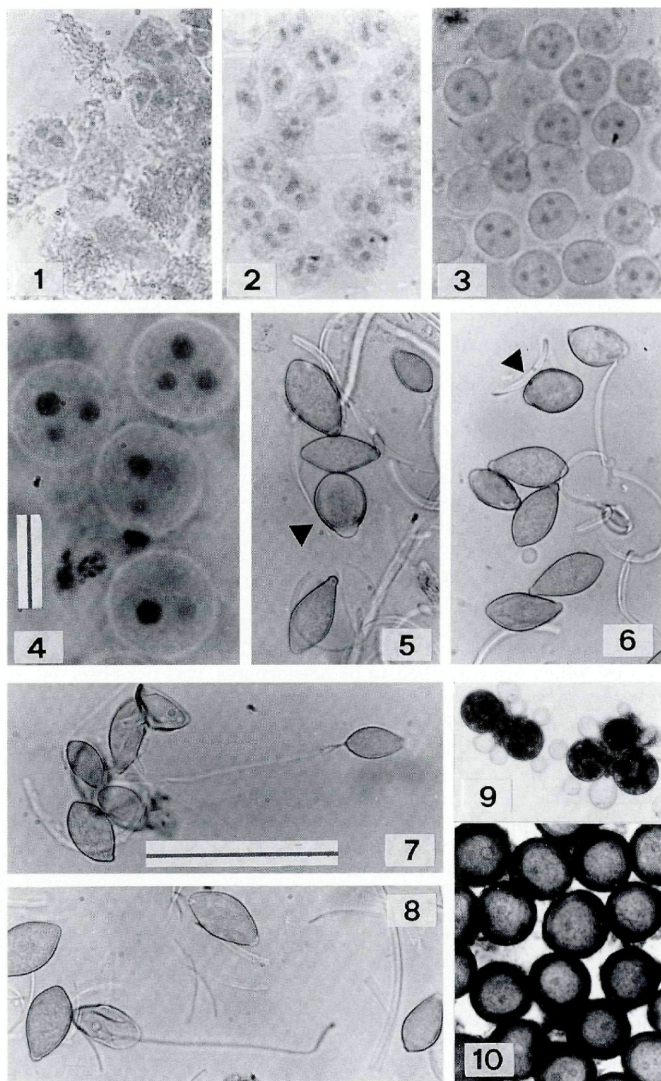
rarely ovoid with broad rounded papilla; smoke-coloured. – C a p i l l i c o n i d i a 13.8–16.3 × 8.5–9.1 μm (11–19 × 7–11 μm), L/D = 1.74–2.13 (7 series), resembling the primary ones, but with the largest diameter in basal half; the outline is more rounded and has a concave detaching point instead of a papilla; tapering towards the apex, mature capilliconidia with apical haptor. – C a p i l l a r y t u b e 46–86 μm (27–140 μm) typically bent near the apical end. – R e s t i n g s p o r e s 17.3–19.4 μm (15–23 μm) (5 series), binucleate, spherical, brown to black, episporium slightly rough to finely granulated (Plate 4, Fig. 3), produced by fusion of two hyphal bodies. The hylum has a diameter of 2.5–3 μm (2 series). – C y s t i d i a , r h i z o i d s and s e c o n d a r y c o n i d i a of type I absent.

Neozygites tetranychi

Type material was made available by J. Weiser. It consisted of a slide with a single *Tetranychus althaeae* (type host) and many dead mites on corrugated cardboard provisionally identified as *T. urticae*. The latter were used for measurements. Primary conidia measure 15.1–16.9 × 12.4–14.3 μm (13–19 × 11–17 μm), L/D = 1.18–1.21 (3 series). Capilliconidia measure 19.5–22.9 × 9.7–11.3 μm (17–28 × 9–13 μm), L/D = 1.94–2.09 (5 series). The capillary tubes have a length of 34.6–39.1 μm (25–61 μm) (2 series, n = 25 each). The resting spores are subspherical to slightly ellipsoid, rarely spherical or short pyriform and light brown to brown. They measure 22.8–25.0 × 21.1–22.1 μm (18–29 × 16–25 μm), L/D = 1.08–1.15 (5 series). Their surface is rough without distinct ornamentation. The formation of resting spores, which are described to be azygospores, was not observed. A few secondary conidia of type I were present.

The dimensions of the primary conidia matches exactly those given in the original description, whereas those of the capilliconida are smaller and those of the resting spores are larger. The species is closely related with *N. floridana* from which it is distinguished by the slightly larger structures, the shape of the resting spores and, in particular, their mode of formation. Further, a single infected mite usually contains both resting spores and conidia, in contrast to *N. floridana*.

Plate 3. – Figs. 1–9: *N. cinarae* sp.nov. from *Cinara pilicornis*. – 1. Hyphal bodies with nuclei. – 2. Hyphal bodies germinating with single germ tube. – 3. Conidiophores, some of them stunted at the host's cuticule. – 4–5. Primary conidia slightly differing in shape. – 6. Capilliconidia on capillary tube and remains of the primary conidia. – 7. Detached capilliconidium with haptor. – 8. Immature zygospores with two nuclei. – 9. Mature zygospores. – Bars in Figs. 5 and 6 = 50 μm; Figs. 1, 4–5, 7–9 and 2–3, 6 same magnification.
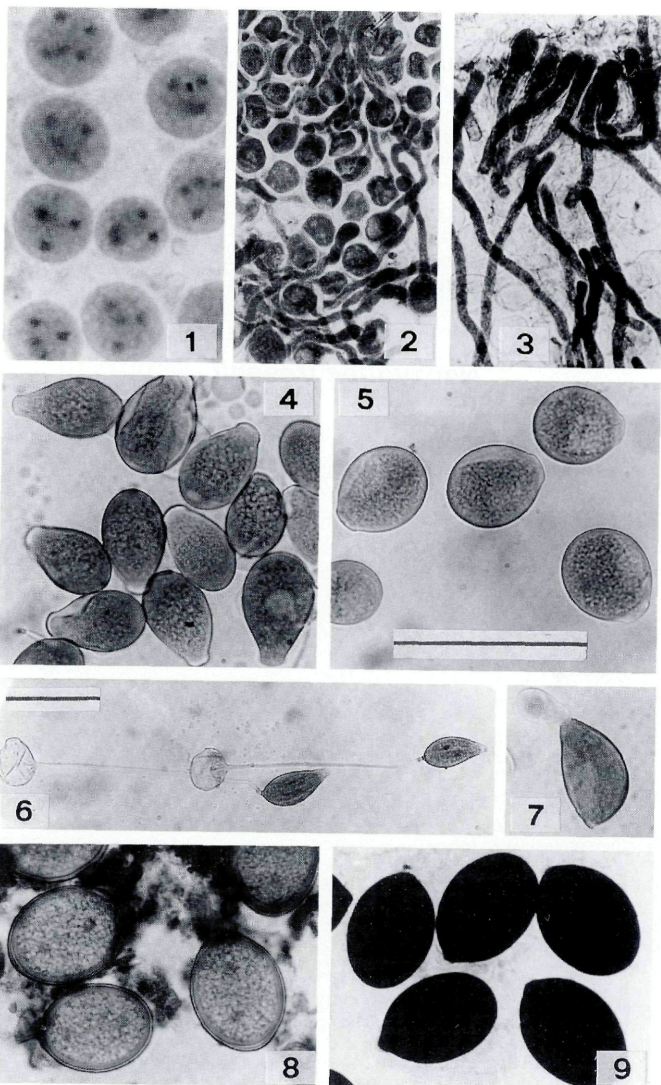
Biology

The material available allowed detailed studies on *N. floridana*, *N. fresenii*, *N. fumosa*, *N. microlophii*, *N. parvispora* and *N. turbinata*. There is strong evidence that *N. floridana* and *N. fresenii* infect only through capilliconidia (Brobyn & Wilding, 1977; Oduor, 1995; Smitley & Kennedy, 1986) which may also be true for other species producing this type of conidia.

The biology of *N. fresenii* and of *N. microlophii* can be considered identical and is as follows (Fig. 1): First sign of an infection are 3-5 nucleate, asymmetrically rounded or comma-like shaped protoplasts (B) which multiply by binary fission (C). In the asexual or
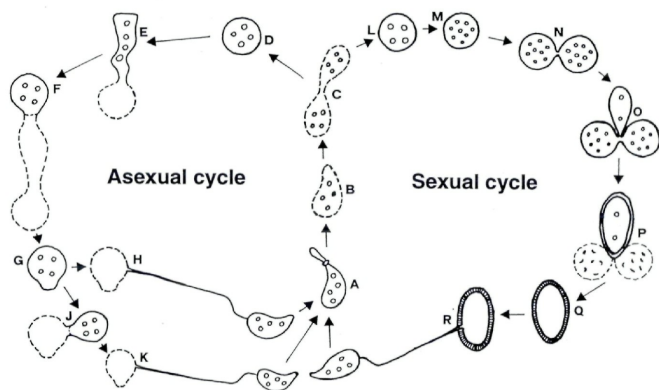
Neozygites fresenii

Fig. 1. – Biology of *N. fresenii*. – A–K: Asexual cycle. – A: Detached capilliconidium with haptor and germ tube to penetrate host's cuticule; B: Protoplast; C: Binary fission of a protoplast; D: Hyphal body; E: Young conidiophore stunting below the host's cuticle; F: Conidiophore after penetration of host's cuticle with primary conidium; G: Projected primary conidium; H: Primary conidium with capilliconidium (secondary conidium of type II); J: Primary conidium with secondary conidium of type Ia; K: Type Ia secondary conidium with tertiary capilliconidium. – L–R: Sexual cycle. – L: Hyphal body; M: Hyphal body with number of nuclei doubled; N: Conjugating hyphal bodies; O: Developing zygospore; P: Young zygospore with adhering shells of hyphal bodies containing degenerating nuclei; Q: Fully developed zygospore; R: Zygospore germinated with a capillary germ conidium. Structures with dotted outline are free of cytoplasm. – The formation of tertiary and higher orders of conidia is omitted.

conidial cycle they develop to spherical hyphal bodies (D) which soon start to form a single unbranched conidiophore (E). The host dies between the stages D and E; its body is filled with ungerminated or germinated hyphal bodies. The developing conidiophores first stunt below the host cuticle (E), but then rupture it by mechanical pressure. The following process takes place outside the host's body: The conidiophores fully develop and form a single primary conidia at the tip (F) which is actively projected. The primary conidia (G) form secondary conidia either on a short, thick conidiophore resembling the primary ones (type Ia) (J) which is projected too, or an almond- or falciform one on a long and slender capillary tube (capilliconidia or secondary conidia of type II) (H), which is passively detached. Type Ia secondary conidia can form either type Ia or type II tertiary conidia (K), but type II secondary can form only type II tertiary conidia. Conidia of higher orders can be produced. Capilliconidia attach to a passing host with their drop-like, sticky end (haptor), detach from the capillary tube (A) and eventually penetrate the host cuticle to restart the infection process.

In the sexual or resting spore cycle the protoplasts develop to 3–5 nucleate hyphal bodies (L) which subsequently double their nuclear numbers (M) and conjugate with a suitable partner body (N). Often two protoplasts remain together and develop into conjugated hyphal bodies with a doubled number of nuclei. From the conjugation bridge formed between the conjugated hyphal bodies the young zygospores develop by budding. As it grows a nucleus of each hyphal body enters and the zygospore becomes binucleate (O). When all cytoplasm has entered the ellipsoidal zygospore a thick cell wall is formed. The nuclei remaining in the shells of the hyphal bodies collapse or disintegrate (P). Usually the host dies at this stage. The mature resting spore is surrounded by a dark brown to black episporium (Q). Generally the resting spores germinate with a single unbranched capillary germ tube forming a capillary germ conidium at the tip. This is able to form at least two further orders of capilliconidia. Capillary germ conidia of any order are supposed to infect new hosts. Photographic illustrations are given by Keller (1991).

The biology of *N. floridana* (Fig. 2) is considered to be identical with that of *N. parvispora*. It differs slightly from that of *N. fresenii* and *N. microlophii*. Protoplasts have never been observed. The first sign of infection is the presence of rod-like hyphal bodies (B, K) or short filamentous structures. In the asexual cycle the hyphal bodies multiply by binary fission (C) until nutrient sources from the host are used and the host dies. Hyphal bodies germinate with a single germ tube which stunt below the host's cuticle (D) and finally rupture it by pressure. Formation of primary (E, F) and secondary conidia (G, H, J) corresponds to that of *N. fresenii*.
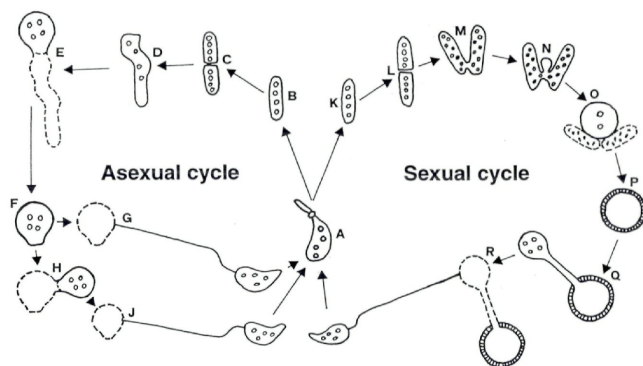
Neozygites floridana

Fig. 2. – Biology of *N. floridana*. – A–J: Asexual cycle. – A: Detached capilliconi-
dium with haptor and germ tube to penetrate host cuticle; B: Hyphal body; C:
Hyphal bodies multiplying by binary fission; D: Young conidiophore stunting be-
low host's cuticle; E: Conidiophore after penetration of host cuticle with primary
conidium; F: Projected primary conidium; G: Primary conidium with capilliconi-
dium; H: Primary conidium with secondary conidium of type Ia; J: Type Ia sec-
ondary conidium with tertiary capilliconidium. – K–R: Sexual cycle. – K: Hyphal
body; M: Conjugating hyphal bodies, number of nuclei doubled; N: Developing
zygospore; O: Young zygospore with adhering shells of hyphal bodies containing
degenerating nuclei; P: Fully developed zygospore; Q: Zygospore germinated with a
rounded germ conidium on thick germ tube; R: Rounded germ conidium with
capilliconidium. – Structures with dotted outline are free of cytoplasm. Only the
4–nucleate variant is drawn. The formation of tertiary and higher orders of conidia
is omitted.

The sexual cycle starts with the conjugation of two rod-shaped
hyphal bodies which obviously induces the doubling of the number
of nuclei (M). The conjugation bridge is formed laterally in the
terminal part. The zygote develops by budding from the conjugation
bridge (N). During its early development a nucleus of each hyphal
body enters (O). The growth of the young zygospore is completed
when all cytoplasm from the hyphal bodies has joined it. Subse-
quently a thick wall is formed and the shells of the hyphal bodies
with the remaining nuclei collapses and disintegrate. The mature
zygospore is spherical to slightly subspherical and surrounded with
a brown to black, slightly (*N. floridana*) or distinctly ornamented *(N.
parvispora)* episporium. Resting spores of *N. floridana* germinate
with a single thick germ tube to form a single rounded primary germ

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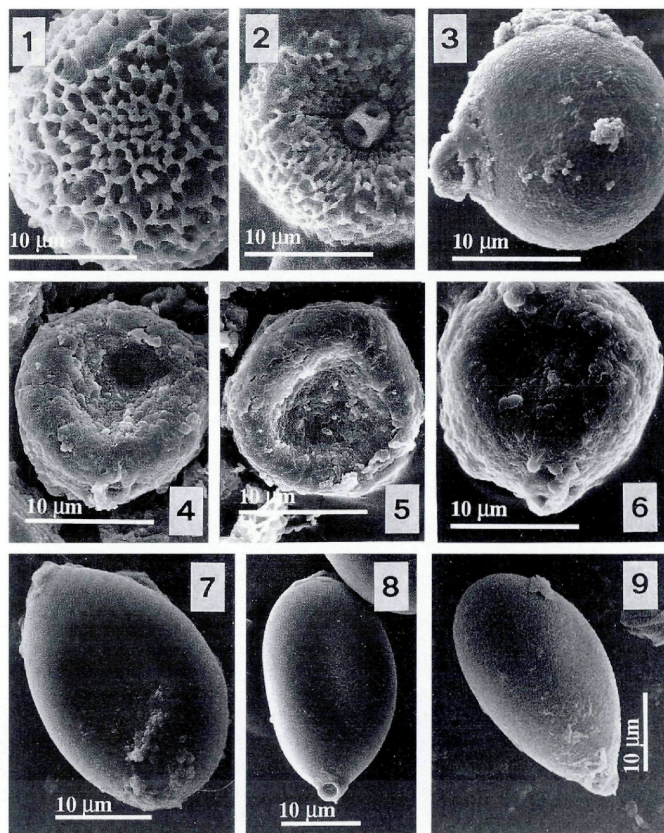
Plate 4. – Figs. 1–9. Scanning electron micrographs of resting spores of *Neozygites* spp. – 1. *N. parvispora.* – 2. Hylum of *N. parvispora* showing the two scars originating from the detached, conjugating hyphal bodies. – 3. *N. fumosa.* – 4. *N. floridana* from *Tetranychus urticae.* – 5. *N. floridana* from *Mononychellus tanajoa.* – 6. *N. tetranychi.* – 7. *N. cinarae.* – 8. *N. fresenii.* – 9. *N. microlophii.* – Magnifications are given in the figures.

conidium. This remains attached and forms a single, capillary secondary germ conidium, which is supposed to infect new hosts. The mode of germination of *N. parvispora* is unknown. Photographic illustrations are given by Keller (1991).

The biology of other known species of this genus can be classified as either the *N. fresenii*-type or to the *N. floridana*-type. There are, however, exceptions. *N. turbinata* lack any type of secondary conidia but the resting spores germinate with capilliconidia (Keller, 1991). *N. tetranychi* forms azygospores (Weiser, 1968) which is unique in this genus and needs confirmation. The fungi from earth and predatory mites and from Collembola are incompletely known and can not be attributed to any of these two life cycles. They probably form a distinct group.

Several steps in the biology are not yet understood. This includes host recognition and specificity, factors determining the asexual or sexual cycle (in an individual host, either conidia or resting spores are formed, *N. tetranychi* being an exception), nuclear processes in the zygospore and during the doubling of the nuclei in the hyphal bodies prior to zygospore formation which halves the volume of the nuclei, and conditions inducing resting spore germination. There are indications that *N. fresenii* might be heterothallic (Villacarlos & Pell, pers. comm.).

The genus *Neozygites*

Neozygites Witlaczil, Arch. f. Mikr. Anat. 24: 599–603 (1885).

Syn.: *Empusa* subg. *Triplosporium* Thaxt., Mem. Boston Soc. Nat. Hist. 4: 152 (1888).
Triplosporium (Thaxt.) Batko, Bull. Acad. Polon. Sci., Sér. Sci. Biol. 12: 324–325 (1964).
Thaxterosporium Ben-Ze'ev & R.G. Kenneth in Ben-Ze'ev & al., Mycotaxon 28: 323 (1987).

Protoplasts spherical to subspherical. – Hyphal bodies regular, spherical or rod-shaped. – Nuclei 3–8 μm, do not stain or stain either poorly or distinctly with LPAO, but stain distinctly with DAPI. – Conidiophores unbranched with more or less distinct terminal enlargement. – Primary conidia unitunicate, spherical, pyriform, ovoid, or in the shape of a hot air balloon, hyaline or light brown, papilla tunicate, cylindrical or conical, small, usually 3–8 nuclei, not or indistinctly staining in LPAO. – Secondary conidia like primary, produced on short lateral secondary conidiophores, or capilliconidia produced on long, slender capillary, amygdaliform to cucumber-like, light brown with terminal droplet or haptor. – Resting spores zygospores produced by conjugation of two hyphal bodies, binucleate with a nucleus from each hyphal body, rarely azygospores, spherical or ellipsoidal; episporium brown or black, smooth, rough or typically ornamented, rarely absent. – Germ conidia correspond to one of the two types of secondary conidia: spherical, hyaline on short thick germ tube or amygdaliform, smoky capilliconidia on long, slender capillary. – Cystidia always absent,

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rhizoids usually absent. – Protoplasts, resting spores, type I and/or type II secondary conidia unknown in some species. – Pathogens of Acari, Collembola, Thysanoptera and Homoptera. – No growth on standard media.

Type species: *Neozygites fresenii* (Nowak.) Remaud. & S. Keller, Mycotaxon 11: 331(1980).

Bas.: *Empusa fresenii* Nowak., Pamietn. Wydz. Akad. Umiej. w. Krakow 8: 171–172 (1883).
Syn.: *Neozygites aphidis* Witlaczil, Arch. f. Mikr. Anat. 24: 599–603(1885).

Key to species

1	Pathogen of mites	2
1*	Pathogen of pterygote or apterygote insects	5
2	Hyphal bodies spherical. On predatory or earth mites (Gamasida and Actinedida)	3
2*	Hyphal bodies rod-shaped, primary conidia $12–17 \times 10–15$ µm; resting spores spherical to slightly subspherical, $19–26 \times 18–24$ µm. On Tetranychidae	4
3	Hyphal bodies 8–11 µm; primary conidia $9–12 \times 5–8$ µm; resting spores spherical, hyaline, 10–13 µm .... *N. acaricida* (1)	
3*	Hyphal bodies 15–20 µm; primary conidia $17–23 \times 13–18$ µm; resting spores spherical, dark brown 14–18 µm .... *N. acaridis* (2)	
4	Primary conidia $12–16 \times 10–13$ µm; capilliconidia $14–19 \times 8–11$ µm; resting spores zygospores, $19–25 \times 15–22$ µm .... *N. floridana* (5)	
4*	Primary conidia 16 $(15–17) \times 13$ (12–15) µm; capilliconidia $25–28 \times 8–11$ µm; resting spores azygospores, $20–23 \times 16–19$ µm .... *N. tetranychi* (14)	
5	Pathogen of Collembola. Hyphal bodies spherical, 12–15 µm; primary conidia $13–15 \times 8–10$ µm. On *Sminthurus viridis* .... *N. sminthuri* (13)	
5*	Pathogen of pterygote insects	6
6	Pathogen of Thysanoptera Thripidae	7
6*	Pathogen of Homoptera	8
7	Hyphal bodies $14–26 \times 6–9$ µm; primary conidia $13–16 \times 11–14$ µm; capilliconidia ovoid to subellipsoid, $17–20 \times 7–10$ µm, L/D = 1.8–2.1; resting spores 15–21 µm, spherical to subspherical, episporium irregularly grooved and ridged, dark brown to black .... *N. parvispora* (12)	
7*	Hyphal bodies $28–40 \times 7–9$ µm; primary conidia globose, 14–16 µm; capilliconidia cucumber shaped, $25–30 \times 8–10$ µm, L/D = 2.5–3.5; resting spores 20–23 µm, spherical to subspherical, episporium unevenly rough, dark brown .... *N. cucumeriformis* (4)	

8 Pathogen of Aphididae 9
8* Pathogen of other Homoptera 10
9 Hyphal bodies spherical to subspherical, 14–17 µm; primary conidia 18–22 × 14–18 µm, predominantly with 4 nuclei; capilliconidia 20–27 × 11–14 µm, L/D = 1.5–2.4; capillary tube on average 17–35 µm, resting spores ellipsoid, 30–41 × 18–23 µm, dark brown to black. On *Aphis* spp., *Rhopalosiphum padi*, *Brevicoryne brassicae* .... *N. fresenii* (6)
9* Hyphal bodies spherical to subspherical, 17–22 µm; primary conidia 24–26 × 18–19 µm with predominantly 5 nuclei; capilliconidia 30–34 × 12–15 µm, L/D = 2.3–2.6; capillary tube on average 150–170 µm; resting spores ellipsoid, 35–43 × 20–23 µm, dark brown to black. On *Microlophium* spp. .... *N. microlophii* (11)
9** Primary conidia 28–32 × 17–22 µm; capilliconidia 29–35 × 16–18 µm. On *Myzocallis coryli* and unidentified species ....
 .... *N. lageniformis* (8)
10 On Lachnidae 11
10* On other Homoptera 12
11 Hyphal bodies spherical to subspherical, 15–24 µm; primary conidia 19–23 × 12–17 µm with 8 nuclei on average; resting spores ellipsoid, 32–35 × 21–22 µm, brown to black. On *Pterochloroides persicae* and *Tuberolachnus salignus* .... *N. turbinata* (15)
11* Hyphal bodies spherical 20–22 µm; primary conidia 24–31 × 18–21 µm with predominantly 4 nuclei; capilliconidia 32–34 × 14–17 µm, L/D = 2.0–2.5; resting spores ellipsoid, 34–36 × 23–24 µm, dark brown to black. On *Cinara pilicornis* .... *N. cinarae* (3)
12 On Psyllidae. Hyphal bodies spherical to subspherical, 10–13 µm; primary conidia 22–30 × 9–11 µm, L/D = 2.5–3.0; capilliconidia 17–20 × 8–10 µm. On *Heteropsylla cubana* .... *N. heteropsyllae* (8)
12* On Lecaniidae. Hyphal bodies spherical; primary conidia 18 × 9–10 µm; capilliconidia 12–20 × 5–10 µm; resting spores subspherical to broad ovoid, dark. *On Lecanium viride* ...... *N. lecanii* (10)
12** On Pseudococcidae. Hyphal bodies spherical, 10–12 µm; primary conidia 15–21 × 8–12 µm, capilliconidia 13–17 × 7–9 µm; resting spores spherical, 15–19 µm, dark. On several species of mealybugs .... *N. fumosa* (7)

1. **Neozygites acaricida** (Petch) S. Keller & Milner, comb. nov.

Bas.: *Empusa acaricida* Petch, Proc. Linn. Soc. New South Wales 65: 259–260 (1940).

The species was originally described from the red legged earth mite, *Hylotydeus destructor* (Tuck.), from western Australia. Petch (1940) mentioned two types of primary conidia, an oval one 9–12 × 5–

7 µm and a subglobose one 8 × 6 µm. He observed secondary conida of similar shape, but no capilliconidia. Milner (1985 and pers. comm.) collected the same fungus on two mite species, the original host and *Penthaleus major* (Duges).The resting spores were 10–12 µm, capilliconidia and rhizoids were absent. Material from *Euseius citrifolius* Denmark & Muma collected in Brazil and attibuted to this species had slightly broader conidia (6–8 µm) and spherical hyphal bodies with a diameter of 8–11 µm. The resting spores were 11–13 µm.

2. **N. acaridis** (Petch) Milner, Trans. Brit. mycol. Soc. 85: 641–647 (1985).

Bas.: *Empusa acaridis* Petch, Trans. Brit. mycol. Soc. 27: 87 (1944).

The species was originally found in Great Britain on the predatory mite *Pergamasus crassipes* (L.). Milner (1985) collected the species in Australia on the predatory mite *Macrocheles penetrans* Krantz and provided an amended description. Some structures are still unknown. Resting spores of a fungus collected in Brazil on the phytoseiid mite *Euseius citrifolius* are supposed to belong to this species (Keller, this paper).

3. **N. cinarae** S. Keller, sp. nov. – Pl. 1, Figs. 1–9.

Corpora hyphalia sphaerica (18–)20–22(–25) µm ad subsphaerica, 4(–5) nucleis praedita. Conidiophora simplicia. Conidia primaria (22–)24–31(–35) × (16–)18–22 (–25) µm, pyriformia vel ovoidea, papilla disticta, truncata praedita. Conidia secundaria amygdaliformia (25–)32–34(–41) × (12–)14–17(–23) µm, hyphae capillares evolutae 35–133 µm longae. Sporae perdurantes zygosporae (30–)34–36(–42) × (21–) 23–24(–28) µm, binucleatae, ellipsoideae, nigrae. Rhizoidea, cystidia et conidia secundaria typi I absunt.

In Cinara pilicorni (Hartig) (hospite typico) (Homoptera: Lachnidae).

Holotypus ZT, Zurich-Reckenholz, coll. et leg. S. Keller, VI 1994, no. Cp 74, Paratypi K et BPI.

Rhizoids absent. – Hyphal bodies spherical, 20.5–22(18–25) µm (2 series) or slightly subspherical with usually 4, sometimes 5 nuclei (Fig.1–2). Diameter of nuclei 3.3–3.6(3–4.5) µm (3 series). – Conidiophores unbranched (Fig. 3) with 4, rarely 5 nuclei. – Primary conidia 24–31 × 18.5–21 µm (22–35 × 16–25 µm), L/D = 1.27–1.50 (5 series), ovoid to pyriform, papilla distinct, truncate or slightly rounded (Figs. 4–5). – Capilliconidia 32–34.5 × 13.5–17 µm (25–41 × 12–23 µm), L/D = 1.96–2.52 (4 series), short almond-shaped, brownish, produced on slender capillary 68–108(35–133) µm long (2 series) (Figs. 6–7). – Resting spores 34–36 × 23–24 µm (30–42 × 21–28 µm), L/D = 1.45–1.51 (4 series), ellipsoid, black, produced by fusion of two hyphal bodies (Figs. 8–9), smooth surface (Plate 4, Fig. 7). – Cystidia and secondary conidia of type I not observed.

Host. – Homoptera, Lachnidae: *Cinara pilicornis* (Hartig) (type host).

Symptoms. – Diseased aphids with probosces fixed in the colonies on the underside of braches of young fir trees; brown or black when filled with resting spores.

Distribution. – Switzerland: Watt-Regensdorf (ZH) (type locality).

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

The species was collected in 1991 and 1995 between the end of June and the beginning of July in a plantation of about 10 years old *Picea abies*. It infected nymphs and adults (both alates and apterae).

Neozygites cinarae is closely related to *N. fresenii* and *N. microlophii*. It can be separated from *N. fresenii* mainly by the larger conidia and from *N. microlophii* mainly by the number of nuclei, the dimensions of the resting spores, the length of the capillary tube and the host species. Type Ia secondary conidia were not found. The nuclear number in 4 series of hyphal bodies (50 each) of *N. cinarae* was counted. 60%, 86%, 90% and 98% were 4-nucleate. 88% of conidiophores (1 series) were also 4-nucleate. In *N. microlophii* between 70% and 95% of hyphal bodies and conidiophores were 5-nucleate. The resting spores have the same surface structure as those of *N. fresenii* and *N. microlophii* (Plate 4, Figs. 7–9).

4. **N. cucumeriformis** Miętk. & Bałazy in Bałazy, Flora of Poland. Fungi (Mycota), vol. 24: Entomophthorales: 140–142 (1993).

The species is known only from Poland attacking *Drepanothrips reuteri* (Uzel.) on *Vicia* sp. It is characterised by the typical shape of the capilliconidia.

5. **N. floridana** (Weiser & Muma) Remaud. & S. Keller, Mycotaxon 11: 331 (1980).

Bas.: *Entomophthora floridana* Weiser & Muma, Florida Ent. 49: 155–157(1966).

The species is known from several species of the mite family Tetranychidae. It is a cosmopolitan species and reported from the United States (e.g. Smitley & Kennedy, 1986; Weiser & Muma, 1966), Central and South America including Cuba and Trinidad (Delalibera & al. 1992, Smith, pers. comm.), Europe (Balazy, 1993; Keller, 1991),

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Israel (Kenneth & al., 1972), India (Ramaseshiah, 1971), Japan (Nemoto & Aoki, 1975), China and Indonesia (Smith, pers. comm.) and East and West Africa (Bartkowski & al., 1988; Yaninek & al., 1996). The morphology and biology of the species is well known (Carner, 1976; Keller, 1991; this paper; Selhime & Muma, 1966). Rhizoids in *Neozygites* are only known from this species. They are sometimes formed in the presence of resting spores and are known from two host species, *T. urticae* (Keller & Wuest, 1983) and *M. tanajoa* (Elliot, pers. comm.). Several studies on abiotic factors and on epizootiology have been reported. The literature has recently been reviewed by Oduor (1995). It is considered to be an important mortality factor and able to induce epizootics especially among the oligophagous common spider mite *T. urticae* and the monophagous green cassava mite *Mononychellus tanajoa* (Delalibera & al., 1992; Klubertanz & al., 1991; Smitley & Kennedy, 1986).

6. **N. fresenii** (Nowak.) Remaud. & S. Keller, Mycotaxon 11: 332 (1980).

Bas.: *Empusa fresenii* Nowak., Pam. Akad. Umiej. Krakow 8: 171–172 (1883).

The species was identified from *Aphis craccivora, A. fabae, A. gossypii, A. rumicis, A. urticata, Brevicoryne brassicae, Microlophium evansi, Myzus persicae* and *Rhopalosiphum padi* as well as several unidentified aphids. It is known from all continents including the South Pacific region and considered the most common aphid pathogen in tropical regions (Remaudière, 1977; Remaudière & al., 1981). The morphology and biology of this species is well known (Keller, 1991; this paper; Soper & MacLeod, 1963).

7. **N. fumosa** (Speare) Remaud. & S. Keller, Mycotaxon 11: 332 (1980).

Bas.: *Entomophthora fumosa* Speare, U.S. Dept. Agric. Bull. 1117: 6 (1922).

The species attacks *Pseudococcus citri, Phenacoccus manihoti, P. herreni, Phenacoccus* sp. (Speare, 1922; Le Rü & al., 1985, Delalibera & al. 1996) and *Rastrococcus invadens* (Lomer, pers. comm.). It is known from southeastern USA, Brazil, Central and West Africa. New hosts, *Coccidohystrix insolita* (Green) from eggplant and an unidentified mealybug species from the weed *Sida* sp., are reported from The Philippines (Villacarlos, pers. comm.). The fungus from *C. insolita* from eggplant has smaller structures (Tab. 3), but our present knowledge does not allow it to be considered a distinct species. *Neozygites fumosa* is considered to be a significant mortality factor of economically important mealybugs like *P. manihoti* on cas-

sava (Le Rü & Iziquel, 1990) and *R. invadens* on mango in West Africa (Lomer, pers. comm.).

8. **N. heteropsyllae** Villac. & Wilding, Mycol. Res. 98: 155 (1994).

The species is known only from The Philippines attacking *Heteropsylla cubana* previously introduced from Central America. Its primary conidia are unusually elongate and the number of nuclei varies between 2 and 8. The resting spores are unknown.

9. **N. lageniformis** (Thaxt.) Remaud. & S. Keller, Mycotaxon 11: 332 (1980).

Bas.: *Empusa (Triplosporium) lageniformis* Thaxt., Mem. Boston Soc. Nat. Hist. 4: 169 (1888).

The species is incompletely described. The available data are compiled by Bałazy (1993). It is known from the USA and Chile.

10. **N. lecanii** (Zimm.) Bałazy, Flora of Poland. Fungi (Mycota), vol. 24: Entomophthorales: 139 (1993).

Bas.: *Empusa lecanii* Zimm., Meded. Lands. Plantent. 44: 25–27 (1901).

The species is incompletely described and known only from South East Asia. The available data are compiled by Bałazy (1993).

11. **N. microlophii** S. Keller, Sydowia 43: 82 (1991).

The species differs from the very similar *N. fresenii* by the distinctly larger structures. It was found in Switzerland and Poland (Bałazy, 1993) on *Microlophium carnosum* Buckton *(= M. evansi* Theobald). Some collections identified as *N. fresenii*, especially from larger aphids, eventually belong to this species.

12. **N. parvispora** (D. M. MacLeod & K. P. Carl) Remaud. & S. Keller, Mycotaxon 11: 332 (1980).

Bas.: *Entomophthora parvispora* D. M. MacLeod & K. P. Carl in MacLeod & al. Entomophaga 21: 307–312 (1976).

The species is known from Europe mainly as a pathogen of the economically important *Thrips tabaci* Lind., but also attacks the barley thrip *Limothrips denticornis* Haliday (Nielsen & al., 1996) and other unidentified thrips. Morphology and biology is well known (Keller, 1991; this paper). The resting spores are characterised by a

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striking ornamentation. After detachment the conjugating hyphal bodies leave two clearly visible scars (Plate 4, Figs. 1–2).

13. **N. sminthuri** S. Keller & Steenberg, Sydowia 49(1): 21–24 (1997).

This is the first entomophthoralean species clearly documented as a pathogen of an apterygote insect. It is known only from Denmark from the lucerne flea, *Sminthurus viridis,* in lucerne and clover fields (Steenberg & al., 1996). Morphology and biology is incompletely known.

14. **N. tetranychi** (Weiser) Remaud. & S. Keller, Mycotaxon 11: 332 (1980).

Bas.: *Triplosporium tetranychi* Weiser, Folia Parasitol. (Praha) 15: 115–122 (1968).

The species is known only from the former Czechoslovakia, attacking *Tetranychus althaeae* and *T. urticae.* It is closely related to *N. floridana.* The most striking differences are the presence of azygospores, which are unique in this genus, and the dimensions of the capilliconidia. The confirmation of the presence of azygospores needs fresh material or the demonstration of the presence of a single scar on the hylum. However, the latter was not seen with the available material (Plate 4, Fig. 6). The surface structure of the resting spores is comparable with that of *N. floridana* (Plate 4, Figs. 4–6).

15. **N. turbinata** (R. G. Kenneth) Remaud. & S. Keller, Mycotaxon 11: 332 (1980).

Bas.: *Entomophthora turbinata* R. G. Kenneth, Mycotaxon 6: 381–390 (1977).

This species is known from Israel and Europe. It attacks *Pterochloroides persicae* and *Tuberolachnus salignus.* Secondary conidia of type I and type II (capilliconidia) are unknown; the resting spores, however, germinate with a capilliconidium. Morphology and biology is well known (Keller, 1991).

Discussion

The genus *Neozygites* was recently reviewed by Bałazy (1993). Since then four species have been added so that 15 species are currently recognized. Furthermore, the knowledge on morphology and life cycles of some previously described species was elucidated.

For a time the genus was considered as consisting of a homogenous group of fungi with four nuclei in all structures present in the asexual cycle. Consequently, the discovery of a species with 8 nuclei and an absence of capilliconidia led to the erection of a new genus, *Thaxterosporium,* to include *N. turbinata* (Ben-Ze'ev & al., 1987). However, studies showed that the so-called 4-nucleate species were not restricted to only 4 nuclei but could vary between 3 and 5. There was also variation between host individuals infected with the same species of *Neozygites* within the same collection. *Neozygites floridana,* for example, has predominantly 3-nucleate structures in some infected hosts and 4-nucleate structures in others. This fact may demonstrate differences in strains, ecotypes or subspecies. On the other hand, *N. fresenii* has predominantly 4-nucleate structures while *N. microlophii* has 5-nucleate ones. In the sexual cycle, the latter species has the same number of nuclei as *N. turbinata,* possibly representing a species intermediate between the 4-nucleate group and the 8-nucleate *N. turbinata.* The recently described *N. heteropsyllae* has 2–8 nuclei in the asexual cycle, definitely demonstrating that a separation between 4- and 8-nucleate groups is no longer tenable. Also, the lack of structures such as capilliconidia or resting spores is no longer something extraordinary but a part of the variability within the genus.

Nevertheless, species of this genus can be placed into groups on the basis of their morphology and life cycle. The *N. fresenii*-group is characterised by the presence of protoplasts, spherical to subspherical hyphal bodies, ellipsoidal zygospores and currently also known to have capillary germ conidia (Fig. 1). The following species can be ascribed to this group: *N. cinarae, N. fresenii, N. microlophii, N. turbinata* and probably *N. lageniformis.* Another group is the *N. floridana*-group, defined by the absence of protoplasts (i.e. protoplasts were never found *in vivo*), presence of rod-shaped hyphal bodies, spherical to subspherical resting spores and, as far as is known, globose germ conidia on a thick germ tube producing capilliconidia (Fig. 2). The following species can be ascribed to this group: *N. cucumeriformis, N. floridana, N. parvispora* and *N. tetranychi.*

Neozygites fumosa and *N. lecanii* seem to be closer related to the *N. fresenii*-group than to the *N. floridana*-group. They have, however, spherical to subspherical resting spores and some structures are unknown which makes it impossible at present to attribute them to either group. The position of *N. heteropsyllae* is uncertain due to missing structures, whilst *N. acaricida, N. acaridis* and *N. sminthuri* seem to be closely related but also different from the other members of the genus. Improved knowledge of their morphology, cytology and biology eventually may lead to the erection of a separate genus to include these latter three species.

Given the present state of knowledge of arthropod-pathogenic genera of Entomophthorales, *Neozygites* seems to be the best adapted one to tropical conditions. Five of the 15 species are found in the tropics, 3 species (*N. fumosa, N. heteropsyllae* and *N. lecanii*) are exclusively found in the tropics, although these regions are still poorly explored with respect to species of *Neozygites* and other member of the Entomophthorales.

Recently, species of *Neozygites* have gained attention as natural control agents of the cassava green mite (*Mononychellus tanajoa*) the mango mealybug (*Rastrococcus invadens*) and Thysanoptera including *Thrips tabaci*. Several projects are studying their potential as classical biological control agents or mycoinsecticides (Smith, 1996; Yaninek, 1994; Yaninek & al., 1996; Lomer, pers. comm.). It is still impossible, however, to cultivate species of these genus *in vitro*. There are only a few reports of successful isolations in complex media, but with growth limited to the protoplast or hyphal body stage (Butt & Humber, 1989; Delalibera, 1996; Leite & al. 1996; Smith, pers. comm.; Grundschober, pers. comm.). A report on successful cultivation on egg yolk must be considered doubtful (Kenneth & al., 1972).

The identification of these fungi is a prerequisite for biological control projects, but current methods based on morphology cannot convey information on aspects such as virulence, strain characterisation or origin. Other techniques based on biochemistry and molecular characteristics need to be applied. It is desirable that fungal strains released in biocontrol programs can be identified either by preexisting or introduced genetic markers to follow their fate in the field and to distinguish them from naturally occurring conspecific fungi.

References

Bałazy, S. (1993). Flora of Poland. Fungi (Mycota) vol. 24. Entomophthorales. – Institute of Botany, Krakow, 356 pp.

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Bartkowski, J., M. O. Odindo & W. A. Otieno (1988). Some fungal pathogens of the cassava green spider mites *Mononychellus* spp. (Tetranychidae) in Kenya. – Insect Science and its Application 9: 457–459.

Batko, A. (1964). On the new genera: *Zoophthora* gen nov., *Triplosporium* (Thaxter), gen. nov. and *Entomophaga* gen nov. (Phycomycetes: Entomophthoraceae). – Bull. Acad. Polon. Sci., Sér. sci. biol. 12: 323–326.

Ben-Ze'ev, I., R. G. Kenneth & A. Uziel (1987). A reclassification of *Entomophthora turbinata* in *Thaxterosporium* gen. nov., Neozygitaceae fam. nov. (Zygomycetes: Entomophthorales). – Mycotaxon 28: 313–326.

Brobyn, P. & N. Wilding (1977). Invasive and developmental process of *Entomophthora* species infecting mites. – Trans. Br. mycol. Soc. 69: 349–366.

Butt, T. M. & I. B. Heath (1988). The changing distribution of actin and nuclear behaviour during the cell cycle of the mite pathogenic fungus *Neozygites* sp. – Eur. J. Cell Biol. 46: 499–505.

—— & R. T. Humber (1989). An immunofluorescence study of mitosis in a mite-pathogen, *Neozygites* sp. (Zygomycetes: Entomophthorales). – Protoplasma 151: 115–123.

——, H. C. Hoch, R. C. Staples & R. J. St. Leger (1989). Use of fluorochromes in the study of fungal cytology and differentiation. – Exp. Mycol. 13: 303–320.

Carner, G. R. (1976). A description of the life cycle of *Entomophthora* sp. in the two-spotted spider mite. – J. Invertebr. Pathol. 28: 245–254.

Delalibera, I. Jr. (1996). Produçao "in vitro" de *Neozygites* sp. (Zygomycetes: Entomophthorales) patogeno de *Mononychellus tanajoa*. – Proc. Simposio Controle Biologico, Foz do Iguaçu, Brasil: 171.

——, R. A. Humber, J. M. S. Bento & A. P. de Matos (1996). Infecçao de *Neozygites fumosa* (Zygomycetes: Entomophthorales) em *Phenacoccus herreni* (Hemiptera: Pseudococcidae). – Proc. Simposio Controle Biologico, Foz do Iguaçu, Brasil: 170.

——, D. R. Sosa Gomes, G. J. de Moraes, J. A. de Alencar & W. Farias Araujo (1992). Infection of *Mononychellus tanajoa* (Acari: Tetranychidae) by the fungus *Neozygites* sp. (Entomophthorales) in northeastern Brazil. – Florida Ent. 75: 145–147.

Keller, S. (1987). Arthropod-pathogenic Entomophthorales of Switzerland. I. *Conidiobolus*, *Entomophaga* and *Entomophthora*. – Sydowia 40: 122–167.

—— (1991). Arthropod-pathogenic Entomophthorales of Switzerland. II. *Erynia*, *Eryniopsis*, *Neozygites*, *Zoophthora* and *Tarichium*. – Sydowia 43: 39–122.

—— & T. Steenberg (1997). *Neozygites sminthuri* sp. nov. (Zygomycetes, Entomophthorales), a pathogen of the springtail *Sminthurus viridis* L. (Collembola, Sminthuridae). – Sydowia 49(1): 21–24.

—— & J. Wuest (1983). Observations sur trois espèces de *Neozygites* (Zygomycetes: Entomophthoraceae). – Entomophaga 28: 123–134.

Kenneth, R. G. (1977). *Entomopthora turbinata* sp. n., a fungal parasite of the peach trunk aphid, *Pterochloroides persicae* (Lachnidae). – Mycotaxon 6: 381–390.

——, G. Wallis, U. Gerson & H. N. Plaut (1972). Observations and experiments on *Triplosporium floridanum* (Entomophthorales) attacking spider mites in Israel. – J. Invertebr. Pathol. 19: 366–369.

Klubertanz, T. H., L. P. Pedigo & R. E. Carlsson (1991). Impact of fungal epizootics on the biology and management of the two-spotted spider mite (Acari: Tetranychidae) in soybean. – Environ. Entomol. 20: 731–735.

Leite, L. G., L. Smith, G. J. Moraes & D. W. Roberts (1996). Production of hyphal bodies of the fungus *Neozygites* nr. *floridana* "in vitro". – Proc. Simposio Controle Biologico, Foz do Iguaçu, Brasil: 448.

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Le Rü, B. & Y. Iziquel (1990). Nouvelles données sur le déroulement de la mycose à *Neozygites fumosa* sur la cochenille du manioc, *Phenacoccus manihoti*. – Entomophaga 35: 173–183.

——, P. Silvie & B. Papierok (1985). L'entomophthorale *Neozygites fumosa* pathogène de la cochenille du manioc, *Phenacoccus manihoti* (Hom.: Pseudococcidae) en République populaire du Congo. – Entomophaga 30: 23–29.

MacLeod, D. M., D. Tyrrell & K. P. Carl (1976). *Entomophthora parvispora* sp. nov., a pathogen of *Thrips tabaci*. – Entomophaga 21: 307–312.

Milner, R. J. (1985). *Neozygites acaridis* (Petch) comb. nov.: An entomophthoran pathogen of the mite, *Macrocheles peregrinus*, in Australia. – Trans. Br. mycol. Soc. 85: 641–647.

Nemoto, H. & J. Aoki (1975). *Entomophthora floridana* (Entomophthorales: Entomophthoraceae) attacking the sugi spider mite, *Oligonychus hondoensis* (Acarina: Tetranychidae), in Japan. – Appl. Entomol. Zool. 10: 90–95.

Nielsen, C., T. U. Madsen, J. Eilenberg & H. Philipsen (1996). Natural occurrence of entomopathogenic fungi on thrips (Thysanoptera) in a Danish wheat field. – IOBC/WPRS Bull. 19(9): 166–171.

Nowakowski, L. (1883). Entomophthoreae. Przyczynek do znajomosci pasorzytnych grzybkow sprawiajacych pomor owadow. – Pamietn. Wydz. Akad. Umiej. w. Krakow 8: 153–183.

Oduor, G. I. (1995). Abiotic factors and the epizootiology of *Neozygites* cf. *floridana*, a fungus pathogenic to the cassava green mite. – Ph. D. Thesis, Univ. Amsterdam, 100 pp.

Petch, T. (1940). An *Empusa* on a mite. – Proc. Linnean Soc. New South Wales 65: 259–260.

—— (1944). Notes on entomogenous fungi. – Trans. Br. mycol. Soc. 27: 81–93.

Ramaseshiah, G. (1971). Occurrence of an *Entomophthora* on tetranychid mites in India. – J. Invertebr. Pathol. 18: 421–424.

Remaudière, G. (1977). Sur quelques Aphidoidea de la Polynésie française. – Bull. Soc. Ent. France 82: 151–155.

—— & S. Keller (1980). Révision systématique des genres d'Entomophthoraceae à potentialité entomopathogène. – Mycotaxon 11: 323–338.

——, J.-P. Latgé & M.-F. Michel (1981). Ecologie comparée des entomophthoracées pathogènes de pucerons en France littorale et continentale. – Entomophaga 26: 157–178.

Selhime, A. G. & M. H. Muma (1966). Biology of *Entomophthora floridana* attacking *Eutetranychus banksi*. – Florida Entomol. 49: 161–168.

Silvie, P. & B. Papierok (1991). Les ennemis naturels d'insectes du cotonnier au Tchad: premières données sur les champignons de l'ordre des Entomophthorales. – Coton Fibres Trop. 46: 293–303.

Smith, L. (1996). Classical biological control activities at the International Center of Tropical Agriculture, Cali, Colombia. – Proc. Symp. Biol. Control, Foz do Iguaçu, Brazil, 9.–14. 6. 1996, 414–418.

Smitley, D. R. & G. G. Kennedy (1986). Role of the entomogenous fungus, *Neozygites floridana*, in population declines of the two-spotted spider mite, *Tetranychus urticae,* on field corn. – Ent. exp. et appl. 41: 255–264.

Soper, R. S. & D. M. MacLeod (1963). Spore morphology of *Entomophthora fresenii* Nowakowski. – J. Insect Pathol. 5: 478–482.

Speare, A. T. (1922). Natural control of the citrus mealybug in Florida. – USDA Bull. 1117.

Steenberg, T., J. Eilenberg & J. Bresciani (1996). First record of a *Neozygites* species (Zygomycetes: Entomophthorales) infecting springtails (Insecta: Collembola). – J. Invertebr. Pathol. 68: 97–100.

Thaxter, R. (1888). The Entomophthoreae of the United States. – Mem. Boston Soc. Nat. Hist. 4: 133–201.

Villacarlos, L. T. & N. Wilding (1994). Four new species of Entomophthorales infecting the leucaena psyllid *Heteropsylla cubana* in the Philippines. – Mycol. Res. 98: 153–164.

Weiser, J. (1968). *Triplosporium tetranychi* sp. n. (Phycomycetes, Entomophthoraceae), a fungus infecting the red mite *Tetranychus althaeae* Hanst. – Folia Parasitol. (Praha) 15: 115–122.

—— & M. H. Muma (1966). *Entomophthora floridana* sp. nov. (Phycomycetes: Entomophthoraceae), a parasite of the Texas citrus mite, *Eutetranychus banksi*. – Florida Ent. 49: 155–159.

Witlaczil, E. (1885). *Neozygites aphidis*, eine neue Gregarine. – Arch. f. Mikr. Anat. 24: 599–603.

Yaninek, J. S. (1994). Cassava green mite intervention technologies. – African Crop Science Journal 2: 361–367.

——, S. Saizonou, A. Onzo, I. Zannou & D. Gnanvossou (1996). Seasonal and habitat variability in the fungal pathogens, *Neozygites* cf. *floridana* and *Hirsutella thompsonii*, associated with cassava green mites in Benin, West Africa. – Biocontr. Sci. & Technol. 6: 23–33.

Zimmermann, A. (1901). De dierlijke Vijanden der Koffiecultuur op Java. Meded. Lands. Plentent. 44: 25–27.

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