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Neozygites abacaridis sp. nov. (Entomophthorales), a new pathogen of phytophagous mites (Acari, Eriophyidae)

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Abstract

A new entomopathogenic fungus, described here as *Neozygites abacaridis* n. sp. (Zygomycetes: Entomophthorales), has been found on the mites *Abacarus hystrix*, *Aculodes dubius*, and *A. mckenziei* (Acari: Eriophyidae). It differs from other *Neozygites* species affecting mites by its small, globose primary conidia, short-ovoid, smoky coloured capilliconidia, and very short capillary conidiophores—which are usually not longer than the spore length. This pathogen infected mite individuals in autumn (from mid-August until mid-November) on *Lolium perenne*, *Agrostis stolonifera*, and *Festuca rubra.* It caused 0.5–1% host's mortality in the vicinity of Siedlce (Eastern Poland) and up to 2–8%, on an average in Puszczykowo (Wielkopolski National Park near Poznań), where its prevalence on some plants reached 13%.

Keywords: *Neozygites*; Eriophyidae; Entomophthorosis; Neozygitaceae; New species

1. Introduction

Observations on mite diseases caused by entomophthoralean fungi are scarce and have significant inaccuracies or vague descriptions. Apart from a limited number of *Neozygites* species causing epizootics in populations of spider mites (Keller, 1997; Keller and Wuest, 1983; Miętkiewski et al., 1993; Nemoto and Aoki, 1975; Tsintsadze and Vartapetov, 1976) which have often been described, the great majority of records describe single observations (Bałazy and Wiśniewski, 1982, 1984; Bałazy et al., 1987; Petch, 1940, 1944), which in only a few cases have been confirmed by further rediscoveries (Bałazy, 1993; Keller, 1997; Milner, 1985). This is mostly due to frequent fortuitous discovery of single host individuals affected by these fungi and difficulties in isolating and culturing of *Neozygites* sp. In the 1990s, a research project was undertaken by the Department of Plant Protection at the Academy of Podlasie in Siedlce, Poland, which aimed to determine the fungal disease frequency in phytophagous mite populations, species composition of infective agents and their significance in the regulation of noxious arthropods (Miętkiewski et al., 2000). Among numerous entomopathogenic hyphomycetes collected on phytophagous mites in the agroecosystems near Siedlce (Eastern Poland), an undescribed member of the genus *Neozygites* was also found. According to reviews by McCoy (1996) and Van der Geest et al. (2000) no entomophthoralean pathogens have hitherto been reported from eriophyid mites. Here we formally describe the morphology and occurrence of this fungus.

2. Materials and methods

Dead individuals of the mites *Abacarus hystrix* (Nalepa), *Aculodes dubius* (Nalepa), and *A. mckenziei* (Keifer) (Eriophyidae) showing signs of infection with entomopathogenic fungi were found in the autumn of 1995, 1996, and 1997 on the grass blades of *Lolium perenne* L. and *Agrostis stolonifera* L., in suburban allotment gardens and meadows surrounding the Liwiec River valley near Siedlce. The same fungus was found in

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September 1999 and mid-August to early November 2002 in mites on lawns of *L. perenne* and *Festuca rubra* L. in Puszczykowo near Poznań, situated near Wielkopolski National Park. In this locality, the most abundant material of the discussed fungus was collected and analysed in detail because of its rather common occurrence. Numerous grass samples from many other localities in Poland (Wielkopolska, Pomerania, and Masuria regions), Germany (Southern Bavaria), and France (Parisian Basin), as well as some collections in Great Britain, The Netherlands, and Denmark were examined in 1999–2002, but this fungus was not found. For the isolation and identification of the pathogenic fungi, dead mites, either mummified or covered by emerging hyphae, were transferred under the stereomicroscope from plants to moist chambers to encourage sporulation. When the mycelium became well developed, a sample or a portion of mycelium was transferred onto Sabouraud dextrose agar (SDA) and SDA enriched with egg yolk (SDEYA), but only growth of hyphomycetes was obtained. Special attempts to isolate *Neozygites* mycelium onto SDEYA media by "conidial shower" method were also unsuccessful. For microscopic measurement of fungal structures, material was prepared in lactophenol (LP), lactophenol with aniline blue (LPAB), and in aceto-orcein (AO), according to Keller's (1987) recommendations. Data on mites' mortality rate caused by entomopathogenic fungi including this pathogen were estimated under stereomicroscope, by direct counting of healthy and dead individuals showing more or less typical signs of mycotic diseases. The typical signs are mummification, external sporulation of *Neozygites* or *Hirsutella*-type, and presence of resting spores inside. Prostrate mycelium protruding from, or covering host's cadavers, especially that of *Verticillium* or *Paecilomyces*-type, or recurrent development of identical mycelia in consecutive sample series were treated as less obvious marks suggesting, however, possible mycoses. Mite population on 30 grass blades randomly selected from greater samples of 200–300 plants, served for quantitative estimations. They were collected from a dozen points of 5–30 m^2 surfaces entirely or partly covered by *L. perenne*, sometimes with slight admixture of *F. rubra.*

3. Results and discussion

3.1. Occurrence and taxonomic status

Single mite individuals infected by *Neozygites* sp. appeared from the beginning of September to early November every year among the more numerous cadavers infected by entomopathogenic hyphomycetes (*Hirsutella* spp., *Verticillium* spp.), probably pathogenic *Chrysosporium* sp. and *Ramularia ludoviciana* Minter, Brady and Hall, or covered by the mycelia of saprophytes. The estimated proportion of cadavers infected by this entomophthoralean pathogen at their peak in mid-October did not exceed 3% in any given sample series of the Siedlce locality, while the most abundant *Hirsutella* species caused 45–55% mortality in the hosts' population. However, in some mid-September 1999 collections from Puszczykowo, about 10–13% mortality was observed over several days, but this dropped to only sporadic cases after the first hoar-frost. In the same locality, similar mortality rate persisted with some fluctuations from September 9 to mid-October 2002. Single cadavers filled with resting spores were found from the first days of October to the end of November. In October, the resting spores appeared usually inside a few dead individuals together with more numerous cadavers covered by conidial sporulation. In one case only, 17 individuals with resting spores were found on one grass blade on November 2, 2002. Neither conidial sporulation nor any other external structures of *Neozygites* mycelium appeared on mite specimens filled with the resting spores. Empty "ghosts" of the primary conidia with capillary conidiophores as well as capilliconidia were observed singly or in groups among the hyphae of saprophytic hyphomycetes overgrowing residues of mite cadavers (Fig. 6). This may suggest a possibly greater role of this pathogen in the host's mortality. A number of quantitative estimations of the fungus occurrence in the mite population during the vegetation season showed its rather strongly diversified distribution on about 500 m^2 of the rye grass lawn in Puszczykowo. After recovering single infected mite specimens in the last days of July 2002, its appearance was checked every few days until mid-November. During the period of pronounced appearance of this pathogen, analysis of host's mortality rate was carried out in several samples. The prevalence of fungous diseases was estimated on the basis of cadavers numbers affected by *Neozygites* and *Hirsutella* species in relation to all mite individuals, excluding exuvia and obvious residues after predators' feed. Despite not infrequent cases of relatively great numbers of dead individuals on some grass blades or even on all parts of particular plants, there were numerous leaves with the mites intact or with only single specimens infected. Hence, the ascertained mortality in particular samples seldom attained about 3% (Table 1). However, in autumnal months the fungus often appeared in relatively great numbers on dying or mummified, individuals put in moist chambers for 2–3 days, showing sometimes more than 10% host's mortality. Some cases of simultaneous development of *Hirsutella* and *Neozygites* mycelia in the same host individuals were found. All these results showed an increased level of the mite mortality caused by this pathogen from early September to mid-October. These observations cannot be treated as an element of epizootiological studies, but

Table 1
Prevalence of *N. abacaridis* in *A. hystrix* population of the Puszczykowo post during the period of August–November 2002

Date and plot number	Number of host individuals					
	Alive	Dead (%)				
		With mycelia of			Undetermined causes	Total
		Neozygites	*Hirsutella*	Others		
17 August 1	120	—	—	—	3 (2.44)	3 (2.44)
20 August 1	116	—	3 (2.31)	8 (6.15)	3 (2.31)	14 (10.77)
20/21 August 1/2	1163	1 (0.08)	31 (2.47)	44 (3.51)	16 (1.27)	92 (7.33)
17 August 2	590	11 (1.63)	23 (3.41)	41 (60.08)	9 (1.34)	84 (12.46)
21 August 2	121	1 (0.77)	4 (3.08)	3 (2.29)	2 (1.53)	10 (7.63)
21 August 2	584	17 (2.23)	72 (9.44)	42 (5.50)	48 (6.29)	179 (23.46)
9/12 September 3	1062	116 (7.76)	69 (4.62)	186 (12.45)	61 (4.08)	432 (28.92)
8 October 3	1754	23* (1.02)	102 (4.53)	236 (10.49)	135 (6.00)	496 (22.04)
2 November 3	884	26*(2.19)	76 (6.40)	43 (3.62)	158 (13.31)	303 (25.52)

* 11 and 21 mite individuals filled with the resting spores, respectively, in October and November.

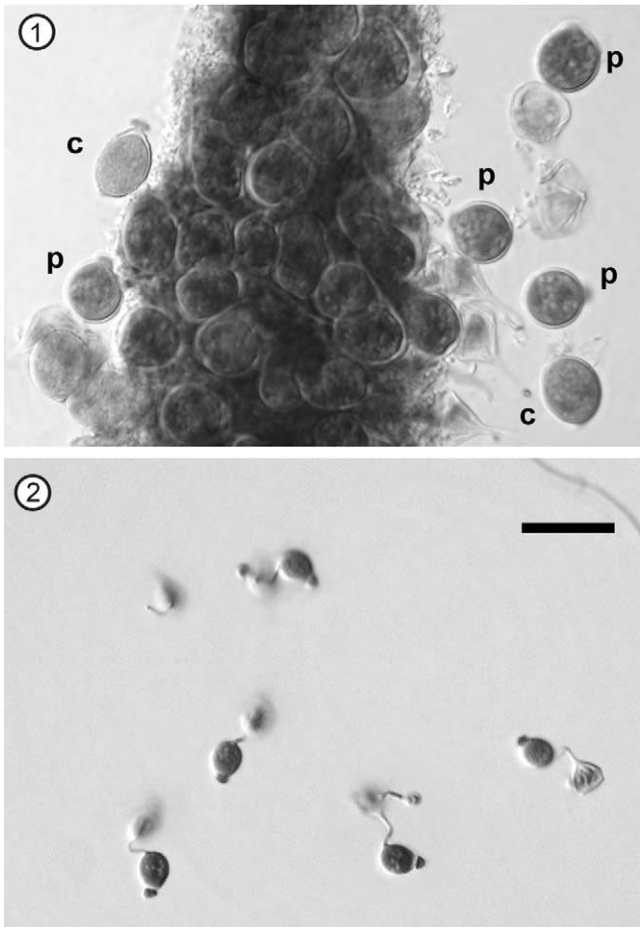
they indicate very uneven distribution of this fungus on the research plot. Despite frequent and thorough searches during all vegetation seasons of 2000 and 2001, this pathogen was not found in afore-described and many other localities, whereas *Hirsutella* infections were common everywhere. Considering a great number of analysed samples gathered in distant regions of Europe, the fungus seems to be rather rare and its effect on grass damaging eriophyids can only be of local significance, as a factor strengthening the mortality caused by *Hirsutella* species.

Species of *Neozygites* have long been known as disease agents of small, delicate arthopods, mostly aphids, thysanopterans, scale insects and mites. Bałazy (1993) listed 11 species in his review and recently four further species have been added. *N. heteropsyllae* Villacarlos and Wilding (1994) has been described from Philippines as a pathogen of the psyllid *Heteropsylla cubana*, which represented a new order of hosts affected by *Neozygites* species. Steenberg et al. (1996) reported an important pathogen of the springtail *Sminthurus viridis* L. in clover and alfalfa cultures in Denmark, named *N. sminthuri* Keller and Steenberg (1997). Continued and developed studies on collembolan mycoses in this laboratory (Dromph et al., 2001) performed data on further new disease agents from the genus *Neozygites* in these insects, but they have not been formally described yet. Keller (1997) separated a new species *N. cinarae* Keller as a pathogen of *Cinara pilicornis* (Hartig) in Switzerland and confirmed the earlier status of *Empusa acaricida* Petch (Milner, 1985) by establishing the combination *N. acaricida* (Petch) Keller and Milner. The best known species *N. fresenii* (Nowakowski) Remaudière and Keller and *N. floridana* (Weiser and Muma) Remaudière and Keller are treated as narrowly oligophagous pathogens of arthropods within the range of one superfamily or family, while others have been reported from single hosts. Hitherto, four species have been known from mites, i.e., *N. floridana* (Weiser and Muma) Remaudière and Keller, *N. tetranychi* (Weiser) Remaudière and Keller, *N. acaricida* and *N. acaridis* (Petch) Milner. Comparing the characteristics of the discussed fungus with other species infecting mites, it is distinguished by clavate hyphal bodies of particular, dorso-ventral arrangement within the host body, very short capilliconidiophores and peculiar short-ovoid and somewhat asymmetrical shape of capilliconidia. The measurements of its primary conidia are much smaller than those of *N. acaridis* reported by Milner (1985), who also characterized the hyphal bodies of the latter as ellipsoid becoming spherical and the resting spores as globose, 14.3–17.9 μm in diameter, of brown wall with finely verrucose epispore. Keller (1997), who examined Brasilian specimens of *Euseius citrifolius* (Denmark and Muma), found almost identical resting spores with "finely pointed ornamentation" and distinctly smaller, spherical hyphal bodies. He cautiously identified this fungus as *N. cf. acaridis.* The mean size of the spherical resting spores of the fungus infecting *A. hystrix* is similar, but there also occur ovoid and short ellipsoid ones and the fine roughness of their surface does not have the character of regular ornamentation comparable with Keller's Fig. 6. The measurements of the primary conidia of the fungus from eriophyids on grasses are very close to those of *N. acaricida* reported by Keller (1997) from *E. citrifolius*, whereas the globose or subglobose hyphal bodies—similarly as resting spores—were significantly smaller. Moreover, any of the above compared fungi produced capilliconidia, whereas in almost all specimens on *A. hystrix* they were the most abundant structures concomitant with sporulation. Because neither morphology nor host range of the here reported fungus corresponds with any of the described species, we consider it justifiable to describe it as a new one under

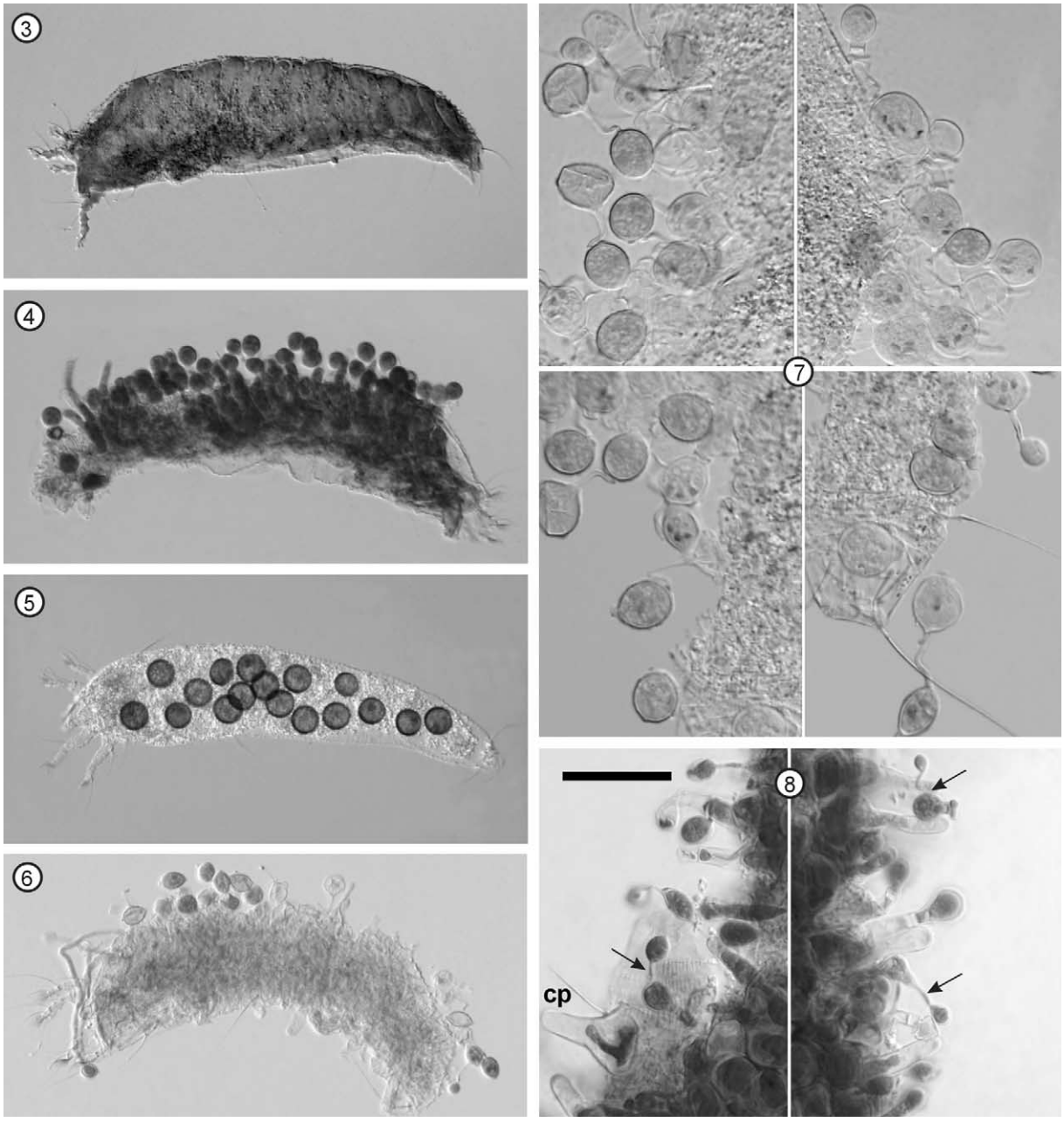
the name *Neozygites abacaridis* n. sp., deriving its specific epithet from the generic name of the predominant host species *A. hystrix*.

3.2. Description

Hyphal bodies causing mummification are generally in a transverse dorsoventral arrangement in the host body cavity, in its anterior and posterior parts obliquely convergent upwards (Fig. 3). However, such an arrangement can only be seen in profile, in early phase of fungus development, before the protrusion of conidiophores. The hyphal bodies are generally clavate, (16–) 17–20 (–24) μm long, (2.5–) 3–4.5 (–6) μm thick at the base, widening upwards up to (4–) 5–7.5 (–8) μm, rounded at tips, typically 4-nucleate, sometimes containing 5–7 nuclei. They protrude from thinner hyphae, 2.5–3.5 μm thick, partly devoid of protoplasm at the moment of investigation, forming a network in ventral layer of host's inside. Singular round or ovoid hyphal bodies of dimensions about 5–12 × 6 μm, 1- to 4-nucleate, have been observed. The nuclei stained in LPAO are round or ovoid, usually 1.5–1.8 μm in diameter. In the sporulation phase the outlines of hyphal bodies are ovoid or elongate, typically 4-, seldom 5-6-nucleate, 5.5–13 × 5.5–10 μm (Fig. 4). The conidiophores protrude dorsally or dorso-laterally, singly from subglobose to broadly ovoid hyphal bodies 9–13 μm of subcuticular layer, as thin-walled, conical outgrowths 10–25 (–35) μm long and 4–5 (–6) μm wide at their bases, gradually tapered to 3.5 or even 2.5 μm at tips (Figs. 4, 8-cp). Primary conidia regularly globose (9.2–) 10–11 (–13.5) μm diameter (Fig. 1), or sometimes ovoid 10–11 × 7.5–9 μm on an average $10.7 \pm 1.06 \times 8.3 \pm 0.99$ μm ($n = 50$). Papillae are slightly convex to almost truncate 1.5–2 μm long and 2–3 μm wide. By the end of the sporulation phase pear-shaped conidia with the basal-part conically elongated towards truncate papilla, and of the total length up to 15 μm (sometimes even longer) often appear. Primary conidia are typically quadrinucleate (Fig. 7) but some have 3 or 5–6 nuclei. Secondary conidia of the ballistospore type were sporadically observed, always much smaller than the primary ones. Typically capilliconidia were produced as the only form of resporulation. Capilliconidia are broadly ovoid, asymmetric when seen in profile, with dorsal outline (see, Bałazy, 1993, p. 25 and Fig. 8) semicircular and the ventral one much weaker convex (Figs. 1c, 2, and 7). Their dimensions together with terminal haptor are (9.5–) 10–12 (–13) × (6.2) 7–8.5 (–9.5), av. $11.3 \pm 0.93 \times 7.6 \pm 0.72$ μm ($n = 50$). Capilliconidia are smoky in colour except a small clear, central spot in the base, and the second at tip around the haptor. In very strong light microscope magnification very small sharp pits scattered sparsely on the surface of darkened capillispore wall are visible. The terminal, asymmetrically located haptor is hyaline, usually 1–1.5 μm long. Capilliconidiophores measured along their curvatures 8.5–12 μm, most frequently 11 μm long and very thin—0.1–0.3 μm, just above half their length and at the end geniculate; they often protrude from the immature primary conidia not yet ejected from the conidiophores especially in early phases of sporulation (Fig. 8, arrows). In damp chambers where grass blades were put directly after sampling, capilliconidia groups were observed on the slides as the only form of resporulation (Fig. 2), helpful for confirming mortality rate in Puszczykowo area. Tertiary capilliconidia occur sporadically and are similar but smaller. Resting spores (Fig. 5) are globose, subglobose, ovoid or short-ellipsoid, blackish or fumose, with amorphous content only weakly transparent under strong microspore light, surrounded by the dark black-brownish wall most frequently 1.2–1.6 μm thick, seldom thinner than 1 μm or thicker than 1.7 μm, of rather very fine, irregular roughness on the surface, without any regular ornamentation. Their dimensions range within the limits (12.5–) 13–17 (–18.2*) μm (see remark in Table 1), both within particular host individuals and time of



Figs. 1–2. Primary conidia and capilliconidia of *N. abacaridis* n. sp. Fig. 1. Primary conidia (p) and capilliconidia (c), bar, 15 μm. Fig. 2. Capilliconidia from the primary conidial "shower" in damp chamber, bar, 28 μm.



Figs. 3–8. Development and morphology of *N. abacaridis*, bar for Figs. 3–6, 50 μm, Figs. 7 and 8, 17 μm. Fig. 3. Dorsoventral arrangement of hyphal bodies within a mummified individual of *A. hystrix*. Fig 4. Conidial sporulation on an infected mite seen in profile. Fig. 5. Resting spores within a dead mite body. Fig. 6. Hyphal and conidial walls and some capilliconidia on residues of host body partly overgrown by saprophytic hyphomycetes. Fig. 7. Nuclei stained in the primary conidia and capilliconidia. Fig. 8. Capilliconidia formation on immature primary conidia (arrows) and protruding conidiophores (cp) in early phase of sporulation.

collection. Only one subglobose resting spore of the size 22.0×21.0 μm was found among 24 filling the host cadaver body (number 9 in Table 2) in the early collection of 8 October 2002. Twenty others, globose in shape were also on average greater than in all the other collections. In one host specimen 9 to about 50 resting spores were observed, most frequently about 20–35. Despite rather abundant materials gathered and examined during the time of the resting spores' appearance (8 October–19 November 2002) no manner of their formation was observed. In some slightly deteriorated cadavers filled with the resting spores some empty residual hyphal body walls were found but it was impossible to determine any traces of conjugation between the hyphal bodies. Numbers of hyphal bodies 2–3 greater per individual during the phase of its mummification seem rather to suggest that resting spore formation is preceded by zygogamy, as in most *Neozygites* species. In the hyla of some resting spores double scars were indistinctly seen which seems to confirm the above supposition.

Table 2
Dimensions of *N. abacaridis* resting spores based on all spores measured in 10 randomly chosen individuals of *A. hystrix*

Current specimen number; its approximate size (μm) and in parentheses number of contained resting spores	Shapes of resting spores and their dimension (μm) with standard deviation (SD)			
	Globose and subglobose (diameter)		Ovoid and ellipsoid (length × width)	
	Variability range	Mean ± SD	Variability range	Mean ± SD
1. 298 × 25–28 (23)	13.0–15.2	14.0 ± 0.49	13.2–15.1 × 10.6–12.1	14.4 ± 0.58 × 11.5 ± 0.69
2. 197 × 26–32 (15)	14.2–16.6	15.3 ± 0.88	15.0–16.4 × 11.2–15.0	15.8 ± 0.50 × 12.8 ± 1.28
3. 176 × 20–35 (18)	13.9–15.2	14.6 ± 0.58	14.2–15.1 × 10.6–13.9	14.8 ± 0.34 × 12.1 ± 1.70
4. 211 × 18–40 (32)	13.0–15.2	13.9 ± 0.53	13.1–16.8 × 9.9–13.8	14.6 ± 0.83 × 11.3 ± 1.10
5. 198 × 33–34 (22)	14.0–16.2	15.0 ± 0.68	14.2–15.6 × 10.3–13.1	15.1 ± 0.40 × 12.1 ± 1.25
6. 165 × 21–35 (16)	13.0–15.2	14.1 ± 0.90	14.0–17.3 × 10.0–12.9	15.1 ± 0.88 × 10.8 ± 1.51
7. 219 × 42–50 (15)	16.0–17.1	16.6 ± 0.58	15.2–18.0 × 11.2–15.5	16.4 ± 0.74 × 13.0 ± 1.30
8. 193 × 18–36 (27)	13.6–15.0	14.2 ± 0.41	13.5–15.5 × 10.0–13.0	14.7 ± 0.67 × 11.2 ± 0.91
9. 218 × 25–26 (24)*	14.9–13.2*	16.0 ± 1.86	14.1–16.8 × 11.8–15.0	15.2 ± 1.37 × 12.9 ± 1.79
10. 233 × 29–50 (45)	12.5–15.1	14.2 ± 0.92	13.5–15.0 × 10.5–13.1	14.4 ± 0.63 × 12.1 ± 0.77
Generalized (231)	12.5–18.2*	14.8 ± 0.92	13.1–18.0 × 9.9–15.5	15.0 ± 0.64 × 12.0 ± 0.78

* One subglobose resting spore of the dimensions 22 × 21 μm has not been considered in the re-count.

The materials were deposited as microscopical preparations in the mycological collection of the Research Centre for Agricultural and Forest Environment of the Polish Academy of Sciences in Poznań. The specimen designated with the number 3556/n was indicated as the holotype (collected in Puszczykowo on 9 of September 2002).

4. Neozygites abacaridis Miętkiewski and Bałazy, n. sp

Fungus entomogenus, in acari Abacarus hystrix, Aculodes dubius et A. mckenziei (Acari: Eriophyidae) parasitans. Hospitis mortui corpus impletus corporibus hyphalibus claviformibus, 16–24 μm longis, ad basim (2.5–) 3–4.5 (–6) μm, in apicibus 4–8 μm crassis, 4- (raro 5–7)-nucleatis. Corpora hyphalia dorsoventraliter disposita, in hospitis corporis finibus sursum oblique convergentia, excrescunt ex hyphis 2.5–3.5 μm crassis, in corporis cavi stratura ventralis textum formantibus. Nonnula corpora hyphalia subglobosa vel ovoidea 1–4-nucleata, dimensionibus 5–12 × 6 μm, videntur. Nucleorum in, ut ita dicam, "aceto-orcein," coloratorum diametro 1.5–1.8 μm. Conidiophora conica, 10–25 μm, raro ad 35 μm, longa, ad basim 4–6 μm crassa, in collum diametro 2.5–3.5 μm attenuata, dorsolateraliter excrescunt e subcuticuli straturae corporibus hyphalibus diametro 9–13 μm. Conidia primaria quadrinucleata, raro 3- vel 5–6-nucleata, regulariter globosa diametro (9.2–) 10–11 (–12.0) μm, vel ovoidea 10–11 × 7.5–9 μm, medio 10.7 ± 1.06 × 8.3 ± 0.99 μm, cum papilla 1.5–2 μm longa, 3–3.5 μm lata, cuius basis leniter convexa est. Extremo sporulationis tempore formantur pauca conidia piriformia, circa 15 μm longa, ad bases truncatas attenuantia. Ballistosporae secundariae pauculae minorae. Capilliconidia fumosa, late ovoidea, dimensionibus 9.8–10.5 × 7.3–8.2 (9.5–) 10–12 (13) × (6.2–) 7–8.5 (–9.5) μm in medio 11.3 ± 0.93 × 7.6 ± 0.72 μm, cum haptore in parte extrema ca 1–1.5 μm longo et 1 μm lato. Imago ex obliquo visa partis dorsalis lineamentum semirotundum, ventralis autem partis superae minus arcuate convexum. Capilliphora 8.5–12 (–14) μm longa, tenuissima (0.2–0.3 μm), extrema in parte semel vel bis inflexa. Capillisporae secundariae non multae, minores. Corpora hyphalia copulata conspectae non sunt. Sporae perdurantes globosae aut subglobosae diametro (12.5) 13–16.5 (–22) μm et ovoideae vel breviter ellipsoideae (13–) 13.5–17 (–18) × (10–) 10.5–15 (–15.5) μm, obscuro fumosae, cum parietibus nigrobrunneis 1.2–1.6 μm crassis, superficie irregulariter ac tenere asperis, medio autumno acarorum mortuorum corpora implent. Holotypus in Abacaro hystrici, ad gramen Lolium perenne, in Puszczykowo prope Posnaniam (Polonia) die 9 mensis Septembris, anno 2002 collectus, in collectione mycologica Instituti Agrariae et Silvestris Oecologiae Academiae Scientiarum Polonorum, Posnaniae, numero 3556/n designatus.

5. Conclusion

Due to the world-scale problem of strong noxiousness of eriophyid mites as vectors of viral diseases in grasslands and cereal crops (Frost and Ridland, 1996; Skoracka and Magowski, 2002; Styer and Nault, 1996), a better recognition of the distribution and role of aforedescribed, most likely specialized pathogen, seems to be justifiable.

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