A new *Nowakowskiella* with yellow-spotted Zoospores — *N. methistemichroma* sp. nov.

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Abstract. — The salient feature of *Nowakowskiella methistemichroma* sp. nov., discovered in Poland, is the stable ability of the refrigent globule of the zoospore to become pure yellow after or during zoospore liberation. *N. methistemichroma* belongs together with *N. moubasheriana* Hassan, to the *N. macrospora*-group, and is the fifteenth hitherto discovered species of the genus *Nowakowskiella*. The key to all known species of this genus included.

Introduction

During the investigation on zoosporic fungi developing on cellulose-containing substrata a sample of water and mud was taken from humid alder forest on the edge of Kampinos National Park near Warsaw. The sample was baited with bits of onion skin and incubated in the laboratory at room temperature. One month later, at the beginning of December 1980, a moderately abundant growth of a rhizomycelial fungus was noticed on some of these baits. The developing fungus produced a loose net of thin rhizomycelial strands sprinkled with numerous spindle organs and sporangia. These was particularly abundant on the edge of the onion skin bits, where it formed dense rows. The more detailed study of this fungus showed that it is an undescribed species of the genus *Nowakowskiella* Schroeter (1893) allied to N. *macrospora* Karling (1945).

Description

Nowakowskiella methistemichroma Batko & Hassan, sp. nov.

Etymology: μεθίστημι χρώματος = this which change the colour.

Rhizomycelium profusum, extensum, ramosum, 2—5 μm diametro, filiforme, isodiametricum, rarenter cum extensionibus plus minusqve sacciformibus, aseptatum, cum organis fusiformibus numerosis. Sporangia plurima, variabilia, plerumque obpyriformia, venter fere sphaericus et collum elongatum habentia, aut elongata, sacciformia vel plus minusqve cylindrica, usque ad 168 μm longa. Zoosporae sphaericae, 8,5—10 μm diametro, cum flagellis 50 μm longis; in zoosporis libere natantiis globuli refractivi lutei 3—3,5 μm

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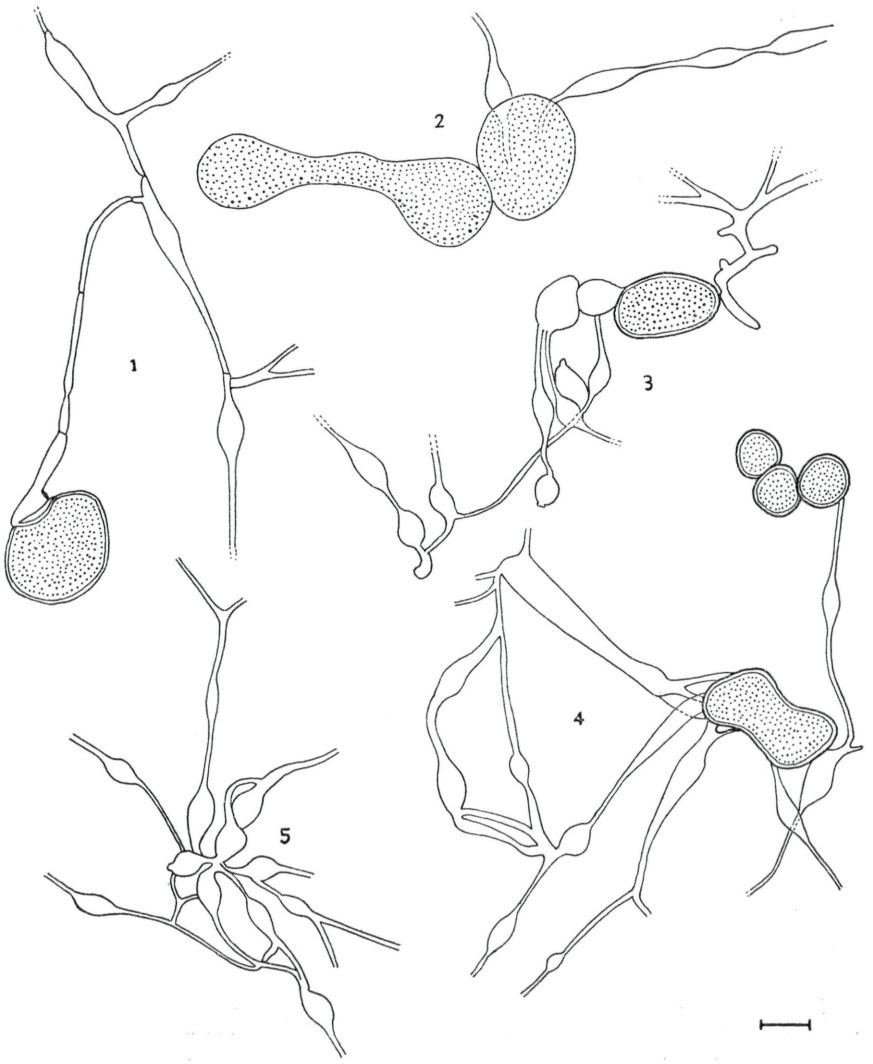
diametro sunt. Sporae perdurantae plerumque subglobosae vel globosae, 28 μm diametro, hyalinae, leves.

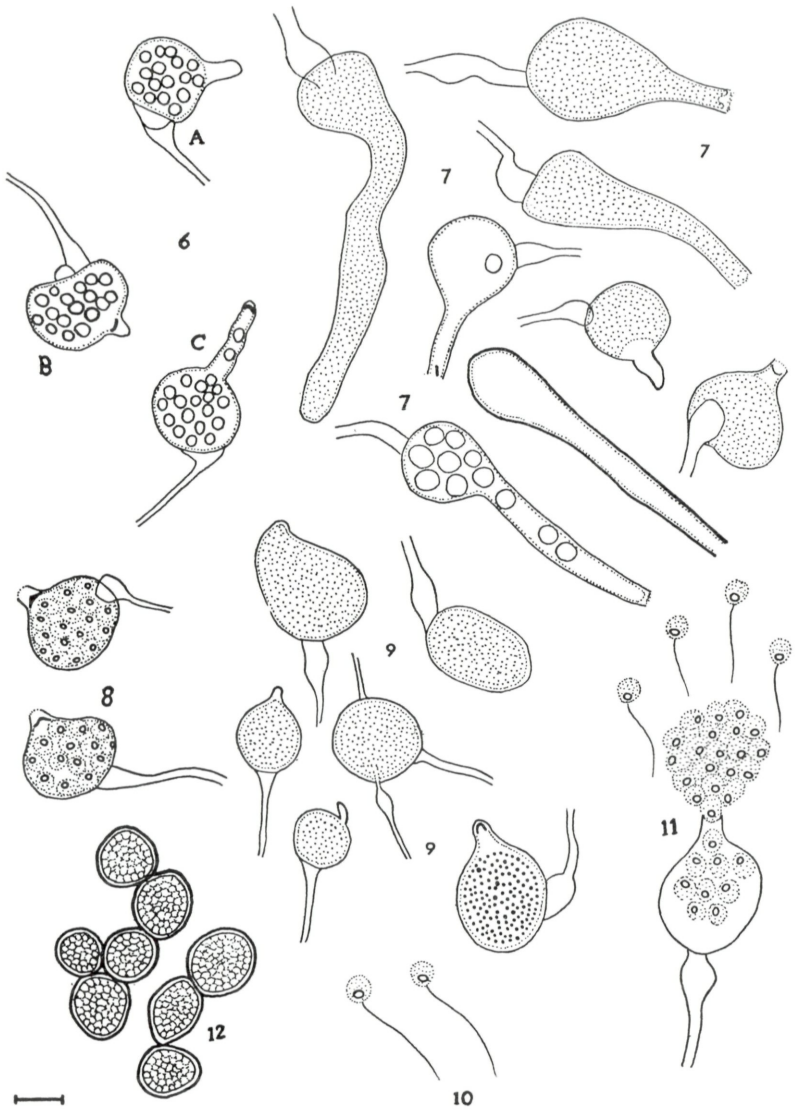
Rhizomycelium profuse, extensive, branched, 2—5 μm in diameter, thread-like and mostly isodiametric (Fig. 1—3), rarely with irregular, elongated, sac-like extensions (Fig. 4), aseptate, with numerous aseptate spindle-like organs 8—22 μm long and 6—12 μm wide, and very thin, scarce rhizoids; wall of the spindle organs and tubular elements slightly thickened and reddish or light reddish-brown; spindle organs mostly regularly distributed along the rhizomycelial strands, but sometimes radially arranged and forming loose clusters (Fig. 5). Sporangia apophysate, abundant, variable in form and size, most often bottle-like (Fig. 6), with nearly spherical base and elongated neck, and then 52—70 μm long including the neck and 36—44 μm wide, or elongated, sac-like to more or less cylindrical (Fig. 7), up to 168 μm long, or with transversely swollen, spheroidal or subspherical basal part (Fig. 6B) up to 34 μm wide and 38 μm high when measured without the neck etc. The neck or evacuation tube usually rather long, up to 84 μm of length, and narrow, 8 μm in diameter; more rarely sporangia with only the shorter, more or less conical papilla (Fig. 8) 7 μm high and 8 μm wide at the base; operculum dome-like, conical, hat-like or in the form of mushroom's pileus, thickened, colourless, 4—8 μm diameter and 1—2 μm high, slightly sunken and usually not protruding above the evacuation tube mouth, formed early in the sporangial development at the stage of evenly granular sporangial content (Fig. 9), pushed out from the tube or papilla before the liberation of the first zoospore (endooperculation A, according to the Dogma's terminology; Dogma, 1973). Apophysis thin-walled, hyaline, most often hemispherical or slightly drop-like, basal or markedly shifted laterally (Fig. 1, 6, 7, 9), 6—12(—22) μm high and (9—)12—22 μm in diameter at the widest part (usually on the plane of contact with zoosporangium). Zoospores (Fig. 10) spherical, 8,5—10 μm in diameter, with long flagellum up to 50 μm and big, mostly more or less central, but often slightly shifted to the flagellum base, refringent globule 3—3,5 μm in diameter, fully formed inside the sporangium and successively liberated; globule of the zoospores inside the sporangium colourless or very slightly greyish, strongly refractive, plastic, changing its shape to rod-like during the movement through the evacuation tube; after liberation zoospore body and globule round up and zoospore rests for the short time (about 30 sec.) motionless, and during this time the colour of globule quickly changes to light but bright lemon-yellow; after activation of the zoospore this colour is more intensified, so that in the older free-swimming zoospore the globule becomes perfectly yellow. First zoospores leave sporangium rather quickly, one after another, passing rapidly through the neck and then form a temporary motionless

cluster at the sporangium mouth (Fig. 11) but the remaining zoospores come through the narrow tube extremely slowly, sometimes during 10 minutes. Resting spores formed in abundance in older cultures, more or less globular, rounded (Fig. 12), hyaline, smooth, 28 μm in diameter.

Habitat: Isolated on onion-skin bait from the stagnant water of the alder forest in Radiowo near Warsaw at the early winter 1981.



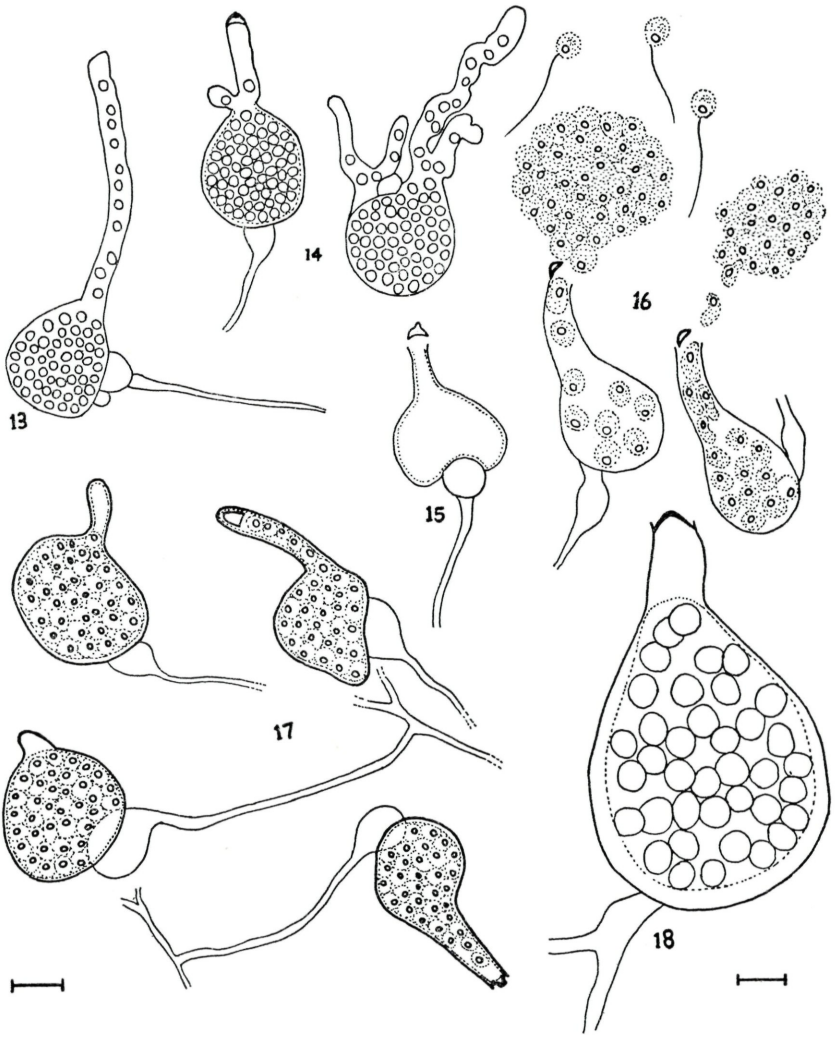
Figs. (1—4). *Nowakowskiella methistemichroma* sp. nov., type strain S 104/81: 1. Rhizomycelium with developing resting sporangium and spindle organs. — 2. Young sporangia. — 3. Branched thallus. — 4. Thallus with developing resting sporangia



Figs. (5—12). *Nowakowskiella methistemichroma* sp. nov., type strain S 104/81: 5. Aggregation of spindle organs. — 6. Nearly mature sporangia at the stage of globule formation. — 7. Sporangia of different shapes and different stages of development. — 8. Ripe sporangia with short necks before the dehiscence. — 9. Younger sporangia showing different stages of globule formation. — 10. Free swimming zoospores with yellow coloured globules. — 11. Zoospores liberation. — 12. Resting spores

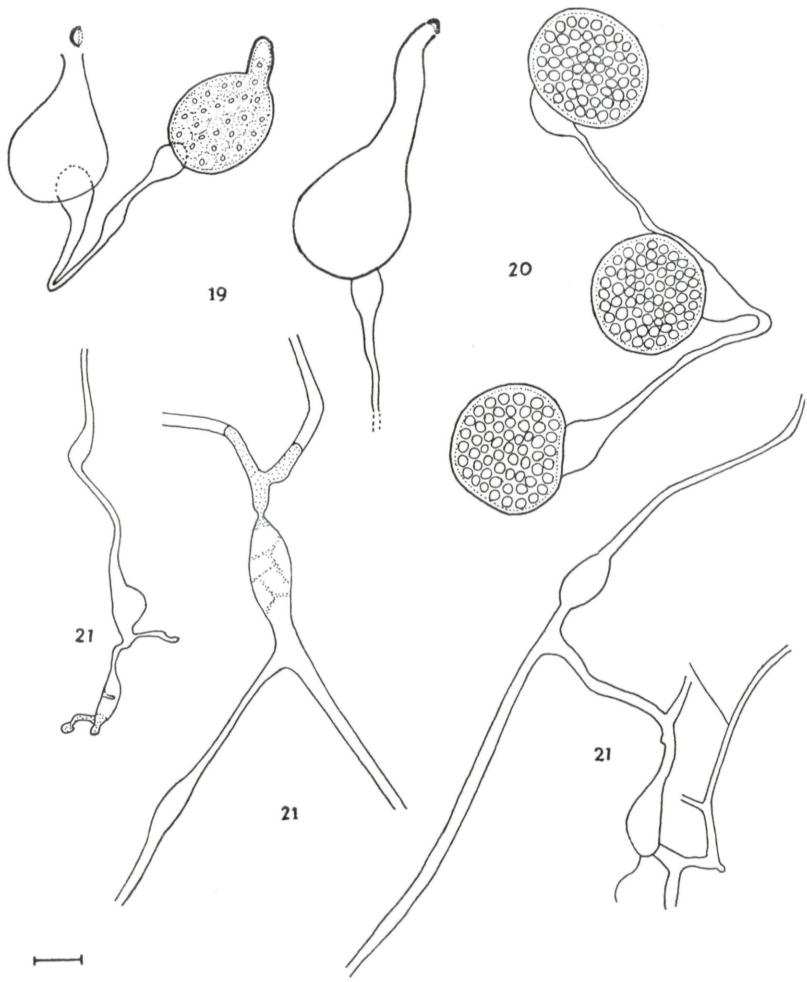
Type: slide no 25704 deposited in the Herbarium of The Institute of Botany, Warsaw University (strain S 104/81); Figs. 1—12.

Apart from the above described type strain, a very similar strain was obtained from another water sample collected at the same time in Dziekanów near Warsaw from a shallow permanent pond on sandy ground in young pine forest. This strain, (S 109/81), differs



Figs. (13—18): Strain S 109/81 of *Nowakowskiella methistemichroma*: 13. Young sporangium with very long neck. — 14. Young sporangia with 2—4 necks. — 15. Empty sporangium with visible cap. — 16. Sporangia liberating zoospores sunken opercula pushed out from the neck. — 17. Apophysate young sporangia. — 18. Sporangium with sunken operculum

from S 104/81 by some minor features, namely: walls of the rhizomycelium were rather dirty-yellowish than reddish, spindle organs are very rare and mostly undistinct, aseptate (Fig. 21), or few thin septa are sometimes formed between alive and dead parts; sporangia are mostly nearly spherical, 52—60 μm in diameter (Fig. 20), with cylindrical neck up to 60 μm long and 11—13 μm in diameter (Figs. 13, 17, 19), or lageniform to elongated, bottle-like, sometimes with more than one neck (Fig. 14), up to 116 μm long and 38—51 μm in diameter,



Figs. (19—21): Strain S 109/81 of *Nowakowskiella methistemichroma*: 19. Sporangial necks of diverse length. — 20. Young apophysate spherical sporangia with sessile caps. — 21. Fragments of young thalli with many spindle organs

Magnifications: Figs. 1—17, 19—21: bar = 20 μm; Fig. 18: bar = 8μm.

very rarely irregular, lobate, in comparison with the type strain with slightly more convex operculum up to 4.5 μm high (Figs. 14—18); some sporangia are exoperculated (Fig. 14); zoospores are bigger, 10—12 μm in diameter. In this strain, globules of zoospores inside the yet closed sporangium are colourless, but begin to turn yellow earlier, even inside the sporangium after its opening. This strain was recognized also as a member of the new species described here.

Discussion

The new species differs from all hitherto known, except *N. macrospora* KARLING (1945), by big spherical zoospores, 8,5—10 μm in diameter in the type strain and 10—12 μm in the second strain. Together with *N. macrospora* and *N. moubasheriana* HASSAN (in press) our fungus constitutes a distinct group of "big spored" species of *Nowakowskiella*. All three species are characterized not only by a considerably big zoospore body, but also by the similar internal organization of the zoospore, i. e. presence of big, plastic refrigent globule. BARR (1980) recently formulated the opinion, that almost all taxonomic criteria used in the systematics of the Chytridiales are now subject to critical reevaluation and tried to create a new classification based on the zoospore ultrastructure. Independently of the evaluation of BARR's proposition concerning the seggregation of the new order Spizellomycetales from the Chytridiales, his view on the zoospore structure as a source of the most valuable taxonomical criteria seems to be right, even on the light microscope level. Apart of that, *N. macrospora*, *N. moubasheriana* and *N. methistemichroma* are similar one to another also in the thallus structure, morphology and general appearance as well as origin and morphology of resting spores. They form, therefore, probably a natural group within the genus *Nowakowskiella*. *N. methistemichroma* differs from both *N. macrospora* and *N. moubasheriana* by its peculiar feature, ability of the zoospore globule to become yellow after or during liberation from the sporangium. Zoospore globules of *N. macrospora* are hyaline and, in the case of *N. moubasheriana*, permanently coloured from the moment of formation inside the ripening sporangium. The colour of the globule of *N. moubasheriana* is yellowish to yellow-greenish, but not pure yellow, as in our fungus. All previously described numerous species of *Nowakowskiella* form colourless zoospore globules, or numerous golden-brown minute granules as in *N. granulata* KARLING (1944). The formation of light yellow zoospore globules has been mentioned as an aberration by WILLOUGHBY (1961) in the case of the English strain of *Nowakowskiella delica* WHIFFEN (1943). In this instance zoospore globules were, however, "sometimes light yellow, otherwise hyaline". In our fungus, as well as in *N. moubasheriana*, the pigmen-

tation of the globule is a constant feature exhibited by all sporangia observed during zoospore liberation in many specimens.

The genus *Nowakowskiella*, created nearly a century ago by SCHROETER (1893), contains now, according to our knowledge, 15 species of these widespread, common, and probably ecologically important fungi. It means, that after the publication of the well-known monograph by SPARROW (1960), six additional species have been described. It seems, therefore, purposeful to publish here a short key based mainly on the characters shown by zoospores.

Key to the species of *Nowakowskiella*

1. Zoospore without larger distinct refractive globule, but numerous golden-brown granules are present . *N. granulata* KARLING (1944)
1*. Zoospores mostly with one single refractive globule (some smaller grains may also be present) 2
2. Refractive globule relatively small, many times smaller than zoospore body 3
2*. Refractive globule relatively large, the diameter of the zoospore body exceeds the diameter of the globule usually 3—5 times .. 7
3. Zoospores up to 4 μm diam. 4
3*. Zoospores over 4 μm diam. 5
4. Sporangia endooperculate; resting spores sculptured .... *N. sculptura* KARLING (1961)
4*. Sporangia exo- or endooperculate; resting spores smooth .... *N. multispora* KARLING (1964)
5. Resting spores yellow-brown; rhizomycelium coarse, septate without distinct spindle organs ... *N. profusa* KARLING (1941)
5*. Resting spores colourless; spindle organs present 6
6. Rhizomycelium fine, thread-like; spindle organs elongated .... *N. delica* WHIFFEN (1943)
6*. Rhizomycelium coarse, tubular, 6—18 μm diam.; with transversely oval to fusoid spindle organs .... *N. crassa* KARLING (1949)
7. Zoospores oval to ovoid; spindle organs and sporangia bearing setae ..... *N. atkinsii* SPARROW (1950)
7*. Zoospores mostly spherical; spindle organs and sporangia smooth 8
8. Zoospores over 8 μm diam. 9
8*. Zoospores mostly under 8 μm diam. 11
9. Refractive globule hyaline, disc-shaped; zoospore over 10 μm diam. ..... *N. macrospora* KARLING (1945)
9*. Refractive globule at least slightly pigmented, yellowish, greenish or pure yellow; zoospores mostly up to 10 μm diam. 10
10. Refractive globule of young zoospore inside the sporangium, colourless, quickly become pure yellow after discharge, lying

in the center of the zoospore body or being slightly shifted ot the base of the flagellum ..... *N. methistemichroma* sp. nov.

10*. Refractive globule yellowish to greenish from the beginning of it existence, lying anteriorly in the zoospore body .... *N. moubasheriana* Hassan (in press)

11. Zoospores 3—3.2 μm diam., with predilection to fatty substrate (e. g. — hemp seeds) and development scarce, if at all, on cellulosic substrata ..... *N. pitcairnensis* Karling (1968)

11*. Zoospores mostly over 4 μm diam.; fungi develop vigorously on cellulosic substrata 12

12. Resting spores formed singly, empty companion cells lacking .. 13

12*. Resting spores formed singly or collectively, in groups of 2—4, inside the thin walled containers, each with empty companion cell 14

13. Sporangia mostly very elongated, often septate, up to 900 μm long ..... *N. elongata* Karling (1944)

13*. Sporangia usually spherical to pyriform, shorter, aseptate .... *N. elegans* Nowakowski (1876)

14. Resting spores formed by proliferation of the external cells of pseudoparenchyma ..... *N. hemisphaerospora* Shanor (1942)

14*. Resting spores formed inside the thin-walled container, each accompanied by the empty accessory cell .... *N. ramosa* Butler (1907)

Acknowledgement

The authors are greaty grateful to Prof. Tomasz Majewski for his kind help in the translation of the formal diagnosis of the new species into Latin.

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