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A new species of *Mortierella*, and an associated sporangiiferous mycoparasite in a new genus, *Nothadelphia*

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Abstract: A new species of *Mortierella* isolated from bat dung in Japan is described as *M. hypsicladia.* The sporangiophores are densely branched in the uppermost part but lack a subterminal vesicle; the species is similar to *M. wolfii* but the terminal sporangium is clearly differentiated being much broader than those borne on the branches; the species also resembles *M. indohii* in having strongly ornamented chlamydospores; it might be a close relative not yet having lost the capacity of sporangiophore formation. A new biotrophic mycoparasite attacking certain *Mortierella* spp. is described in a new genus, *Nothadelphia.* The species produces fascicles of very thin erect sporangiophores, with endogenous many-spored sporangia without a columella, non-motile sporangiospores and thick-walled, papillate chlamydospores. The fungus superficially resembles *Mortierella* or *Umbelopsis*, but its actual phylogenetic position is not yet resolved

Taxonomic novelties: *Mortierella hypsicladia* Degawa & W. Gams sp. nov., *Nothadelphia* Degawa & W. Gams gen. nov., *Nothadelphia mortierellicola* Degawa & W. Gams sp. nov.
Key words: *Actinomortierella, Mortierella, Mucorales,* mycoparasite, taxonomy.

INTRODUCTION

During our studies on Japanese Mortierellaceous fungi, we isolated many species of the genus *Mortierella* from bat dung samples collected in caves. In such locations, *Mortierella alpina, M. indohii, M. polycephala, M. reticulata, M. tuberosa*, etc. were usually abundant. An undescribed *Mortierella* species was repeatedly isolated which we describe here as a new species. In addition, about one week after plating bat dung on agar, bundles of hyaline delicate sporangiophores emerged on the surface of the dung. The sporangiophore bundle radiated from white dense hyphal masses which included many scattered chlamydospores of the above *Mortierella*. Globose, many-spored sporangia appeared on very thin, unbranched sporangiophores. Although the spores never grew out when transferred singly on shrimp, brain-heart infusion, malt-yeast extract, or cornmeal agars, they germinated when coinoculated with a *Mortierella* sp. Finally, two-membered cultures were established using a *Mortierella* sp. from the original dung as a host, and sporulation of the mycoparasite was reproduced under these conditions. This mycoparasite could not be assigned to any other known genus. We propose a new genus to accommodate it.

MATERIALS AND METHODS

Field-collected dung samples of bats (order *Chiroptera*) were directly inoculated on the surface of Sh3A (0.3 % shrimp agar: 3 g dried flaked shrimps (*Sergestes luscens*), 15 g agar, 1 L distilled water, Degawa & Tokumasu 1997). Isolation was carried out using Sh3A and LCA (Miura-agar; Miura & Kudo 1970; Sugiyama *et al.* 2003). Cardinal temperatures for growth were determined on 2 % malt extract agar with incubation at 3 °C intervals. To establish a two-membered culture of the mycoparasite, spores were picked up with Elgiloy needles and inoculated on many points on Sh3A with a range of *Mortierella* species as possible hosts. Cultures were incubated at room temperature (*ca.* 25 °C) or at 20 °C in an incubator. Light-microscopic observation was carried out using BX60, Olympus, with Interference Contrast (DIC) equipments. Slide material was mounted in water or lactic acid (99 %), sometimes with aniline blue staining. Phloxine staining was also applied with 5 % KOH for the observation of details.

RESULTS

Mortierella hypsicladia Degawa & W. Gams, **sp. nov.** MycoBank MB500161. Figs 1, 2.

Etymology: *hypsi* (Greek) = at the top, *clados* (Greek) = branch.

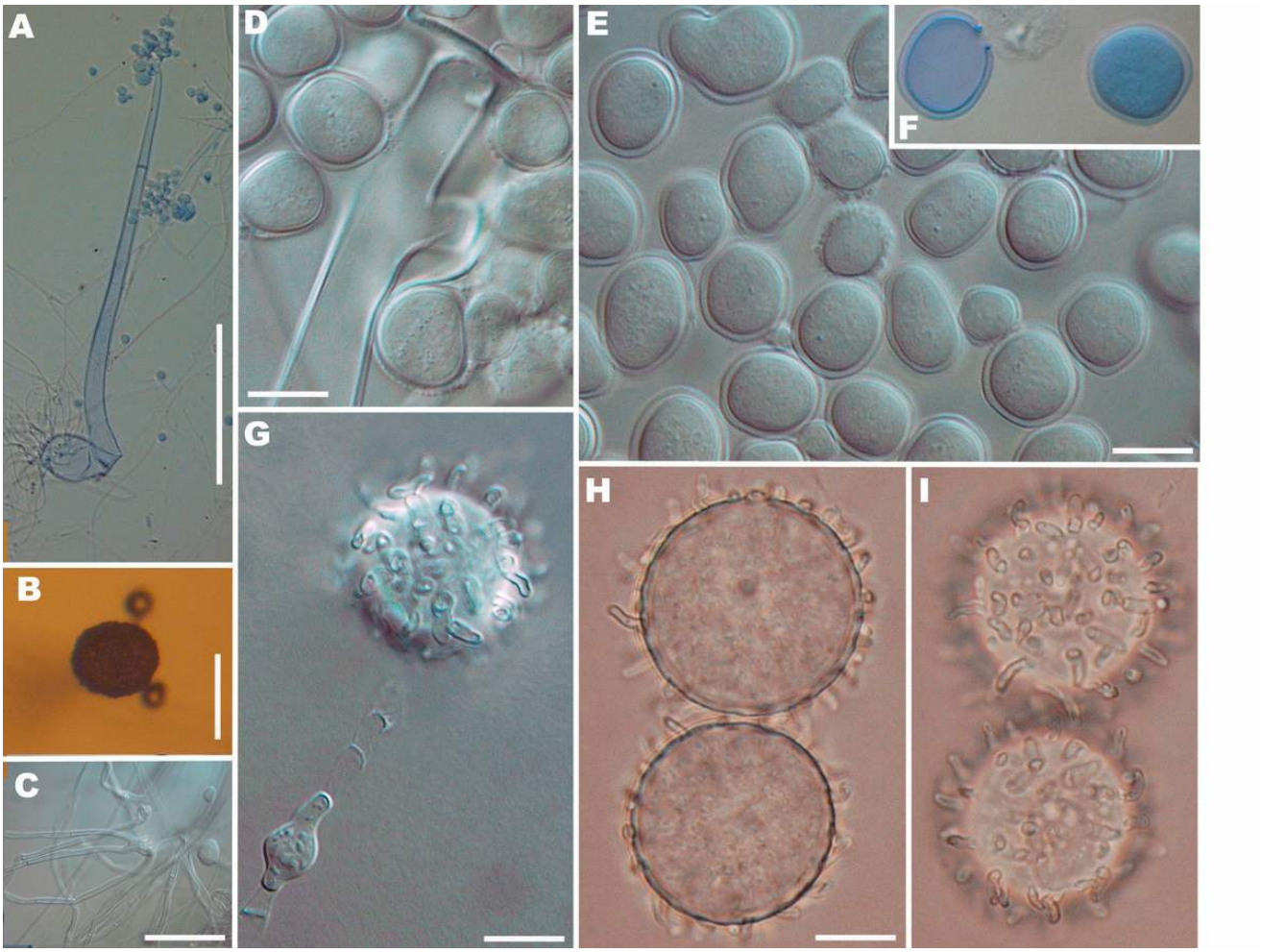


Fig. 1A–I. *Mortierella hypsicladia*. A. Sporangiophores produced on agar plate (Sh3A). B. Sporangia. C. Rhizoid of sporangiophore seen from above. D. Uppermost branchings of sporangiophore. E. Sporangiospores. F. Sporangiospores crushed and stained in lactic acid aniline blue, showing double membranes. G–I. Hairy chlamydospores, the same position with different focusing. Scale bars: A = 200 μm, B = 50 μm, C, D, E–F, G, H–I = 10 μm.

Coloniae celeriter crescentes, interdum zonatae, olentes. Sporangiophora erecta ex agaro oriunda, ad basim rhizoideis praedita, 550–1000(–1800) μm longa, e 25–35 μm ad 7–11 μm sursum angustata, in summo sub apice 9–14 μm lato repetite racemose ramosa, 2–4 ramos primarios, 20–35(–100) × 5–10 μm, et nonnumquam nonnullos secundarios ferentia. Sporangia hyalina, 30–50 μm diam, multispora, collare conspicuum relinquentia. Sporangiosporae late ellipsoideae, laeves, 9–12(–18) × 7.5–10(–12) μm. Chlamydosporae globosae, fimbriatae, fimbriis exclusis 22–32 μm diam. Zygosporae ignotae.

Colonies fast growing, producing a concentric pattern on 2.5 % MEA, well sporulating on Sh3A, with *Mortierella*-like odour. *Sporangiophores* arising from the agar surface with finely branched rhizoids, erect, acrotonously branched in the uppermost part, up to 550–1000 (–1800) μm tall, tapering from 25–35 μm to 7–11 μm near the tip; the terminal, largest sporangium with a 9–14 μm wide stalk, immediately below bearing 2–4 racemose branchlets, 20–35(–105) μm long, 5–10 μm wide, sometimes bearing two or three additional secondary branches. *Sporangia* hyaline, 30–50 μm diam, many-spored, with deliquescent wall, after spore liberation leaving a conspicuous collarette. *Sporangiospores* subglobose, smooth-walled, 9–12 (–18) × 7.5–10(–12) μm. *Chlamydospores* terminal or intercalary, formed within the agar or on the surface, subglobose, bearing numerous 4–5 μm long hair-like projections, without the fimbriae 22–32 μm diam. *Zygospores* unknown.

Temperature requirements: Optimum 21–27 °C, with daily radial increments of 6–8 mm, maximum around 30 °C, no growth at 33 °C.

Specimens examined: **Japan**, Kariu-cave, Ooita pref., Kyushu-island, on bat dung, 3 Nov. 2002, coll. Y. Degawa, KPM-NC0012406 **holotype**; ex-type culture deposited as CBS 116202 (no. 1). KPM-NC0012407, culture CBS 116203 (no. 2) from the same origin, but different sample. The same species was also collected from bat dung in Hakurendo cave, Shimohei, Iwate, 3 Jun. 1993; soil near Rashomon cave, Atetsu, Okayama, 29 May 1994; bat dung in Tohrohkutsu cave, Dorokawa, Nara, 7 May 2001; and Oniana-cave, Abukuma, Fukushima, 12 Aug. 2003.

Note: The species seems to prefer bat dung or soil in limestone areas.

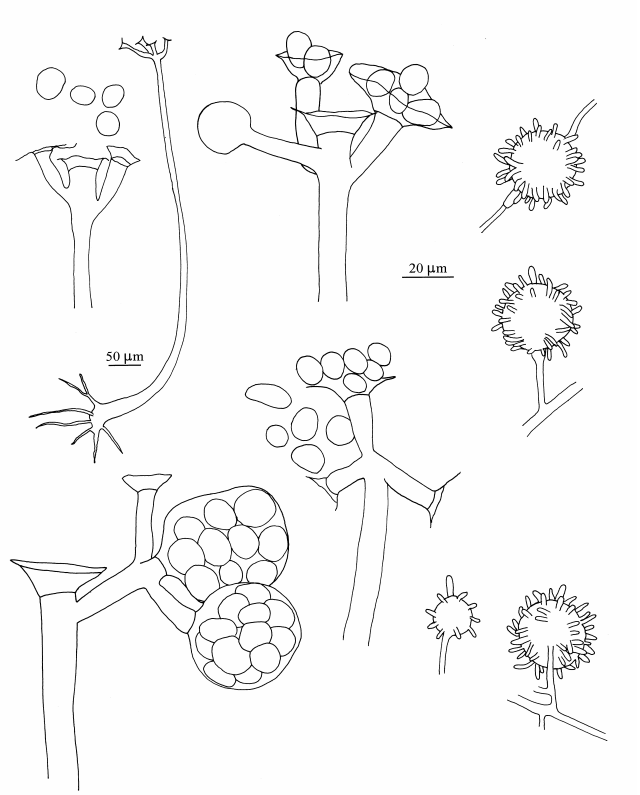


Fig. 2. *Mortierella hypsicladia*, an entire sporangiophore, sporangiophore tips and spores, chlamydospores. Scale bars: A = 50 µm for the single sporangiophore, B = 20 µm for remaining structures.

Nothadelphia Degawa & W. Gams, **gen. nov.** MycoBank MB500162.

Etymology: *nothos* (Greek) = false, *adelphos* (Greek) = brother (Terentius's title of a comedy), referring to false brotherhood among related taxa (adelphoparasitism).

Coloniae albidae, biotrophicae, haustoriis hospitem contingentes. Hyphae vegetativae, delicatae, nonnumquam septatae, parce ramosae. Sporangiophora fasciculata, erecta, numquam ramosa, ex vesiculis basalibus oriunda. Sporangia hyalina, multispora, primo fusiformia, deinde sphaerica, columella carentes, collare parvum relinquentia. Sporangiosporae hyalinae, laeves, cylindraceae vel forma irregulares, paulatim dilatatae vel sphaericae, guttis praeditae, germinantes haustoria formantes. Chlamydosporae intercalares, hyalinae vel pallide flavescentes, modice crassitunicatae, verrucosae, hyphas breves terminales ferentes. Zygosporae ignotae.

Colonies white, densely crowded, haustorial biotrophic on the host fungus. Vegetative mycelium delicate, septate at irregular intervals, scarcely branched. *Sporangiophores* fasciculate, erect, unbranched, arising from basal vesicles. *Sporangia*, hyaline, multispored, fusiform when young, at maturity spherical, with deliquescent wall, without columella, after dehiscence leaving a minute collarette. *Sporangiospores* hyaline, cylindrical to irregularly shaped, some swollen to almost globose, prior to haustorium formation showing oily droplets. *Chlamydospores* hyaline to slightly yellow, thick-walled, papillate, intercalary, usually bearing a free short distal hyphal segment.

Typus: *Nothadelphia mortierellicola* Degawa & W. Gams, sp. nov.

Nothadelphia mortierellicola Degawa & W. Gams, **sp. nov.** MycoBank MB500163. Fig. 3.

Coloniae albidae, in hyphis *Mortierellae* crescentes. Hyphae vegetativae delicatae, 1.5–3 µm latae, aliquando septatae, parce ramosae. Sporangiophora hyalina, fasciculata, erecta ex vesicula basali, 16–23 µm diam, oriunda, stricta, numquam ramosa, tenuitunicata, 140–200 (ad *ca.* 900) µm longa, omnino 1.5–2.4 µm lata, nec rhizoideis nec stolonibus praedita. Sporangia hyalina, primo fusiformia 12–15 × 7.5–9 µm, deinde sphaerica, 17–20 diam, multispora, *ca.* 60 sporas continentia, columella absente, collare parvum relinquentia. Sporangiosporae cylindricae, nonnumquam curvatae vel forma irregulares, 5.5–7.5(–10) × 3–4.5 µm, germinando guttas oleaginosas continentes. Haustoria hyphas hospitis contingentia, 3–8 × 0.8 µm, ante tubum germinativum formata. Chlamydosporae hyalinae vel dilute flavidae, fere crassitinucatae, 16–22 µm diam, verrucis hebetibus obtectae, intercalares. Zygosporae ignotae. Biotrophica hyphas *Mortierellae* specierum parasitans.

Colonies white, densely crowded, growing only on vegetative hyphae of the host fungus. Vegetative hyphae delicate, 1.5–3 µm wide, sparsely septate, sometimes branched. *Sporangiophores* hyaline, erect, fasciculate, on abnormally crowded, dichotomously branched hyphal masses of the host fungus, one to several arising from a basal thin-walled spherical vesicle, 16–23 µm diam, straight, unbranched, thin-walled, about 140, but up to *ca.* 900 µm high, 1.5–2.4 µm wide throughout, without rhizoids or stolons. Vesicles initially fusiform, 12–15 × 7.5–9 µm, to spherical, 16–23 µm diam, with a subvesicular septum. Sporangia hyaline, obovoid when young, at maturity spherical, 17–20 µm diam, multispored (*ca.* 60 spores in a sporangium), with deliquescent wall, without columella, but often leaving a minute collarette. *Sporangiospores* cylindrical, 5.5–7.5(–10) × 3–4.5 µm, sometimes curved to irregularly shaped, sometimes strongly swollen to almost globose, prior to germination containing one to several large refractive oil droplets. *Haustoria* formed directly from swollen spores before germination near the vegetative hyphae of the host, one to three from a spore, minute, 3–8 × 0.8 µm. *Chlamydospores* hyaline to pale yellow, thick-walled, 16–22 µm diam, sometimes covered with blunt warts, intercalary, often bearing a short distal hyphal segment, 18–30 µm long. *Zygospores*

unknown. A haustorial mycoparasite on vegetative hyphae of *Mortierella*.

Specimens examined: **Japan**, Kariu-cave, Ooita pref., Kyushu-island, on bat dung, 3 Nov. 2002, coll. Y. Degawa, KPM-NC0012408, **holotype**; ex-type culture growing on *Mortierella hypsicladia*, deposited as CBS 116204. Known only from the type collection.

DISCUSSION

Mortierella hypsicladia shows an extreme acrotonous sporangiophore branching and may thus be related to *M. wolfii* B.S. Mehrotra & Baijal (1963), which Gams (1970) with hesitation placed in *Mortierella* sect. *Spinosa*. That species differs from the present one by smaller sporangiophores, smaller, distinctly double-walled sporangiospores (6–10 × 3–5 µm), chlamydospores having irregular projections, and much higher cardinal temperatures. The spores of *M. hypsicladia* appear double-walled only when mounted in lactic acid, in water mounts they show a single wall layer. We cannot preclude the possibility that both these species are related to *Actinomortierella* (Degawa & Tokumasu 1997).

Almost identical chlamydospores with hair-like projections (also called "stylospores", Linnemann 1941), are known in *M. indohii* C.-Y. Chien (in Chien *et al.* 1974), which could not be assigned to a section because of absence of sporangiophores. But the zygospores of *M. indohii* obviously document its affinity to *Mortierella* and not *Actinomortierella*. *M. hypsicladia* may be its closest relative that has not yet lost the capacity to form sporangiophores. The present species shows a pronounced differentiation between the largest, terminal sporangium and the smaller sporangia borne on branchlets. This would plead for placing the new species in *Actinomortierella* in spite of the missing terminal swelling. A more accurate classification will become possible only with the analysis of DNA sequences.

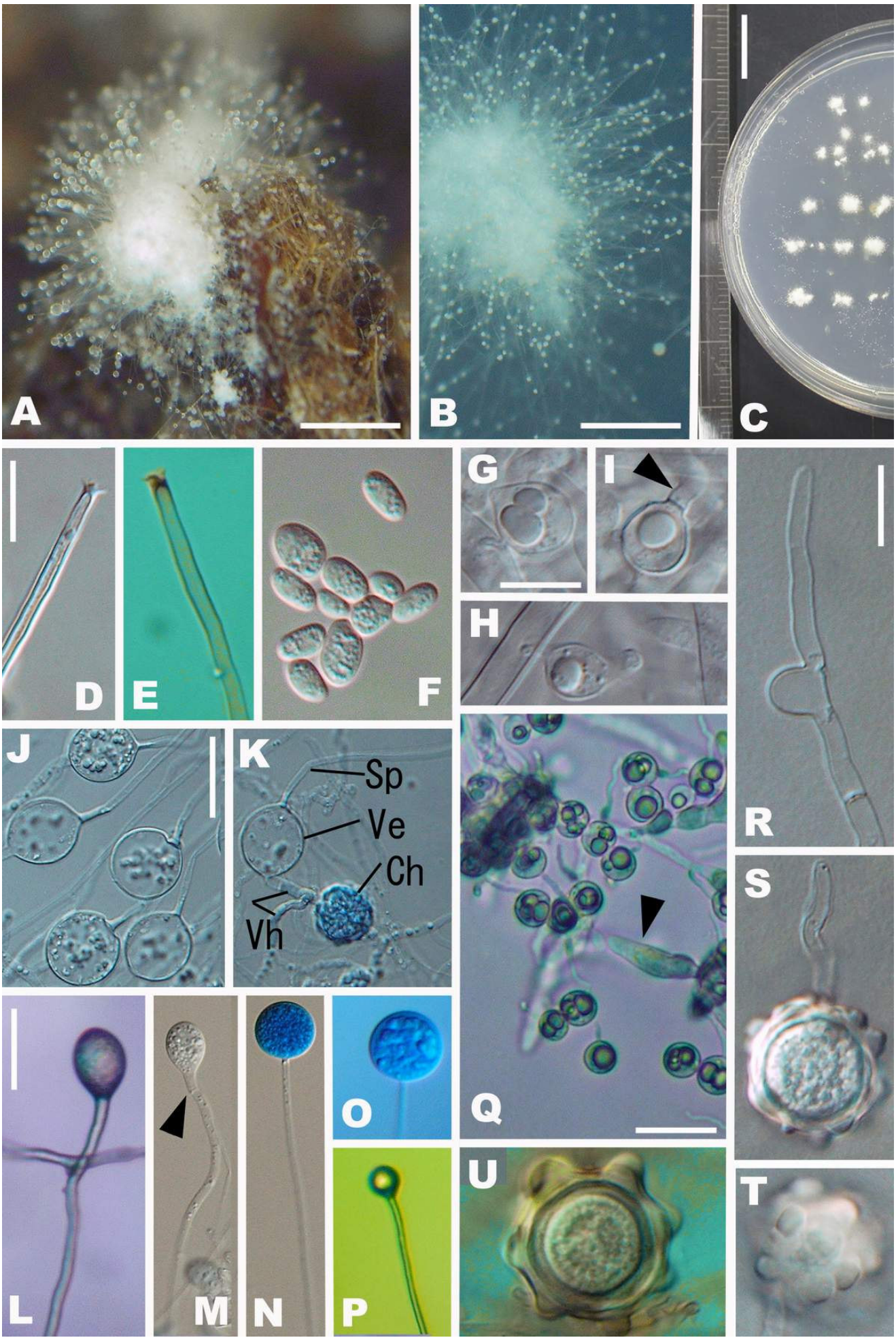
Nothadelphia mortierellicola shows the best growth and abundant sporulation when grown on *M. hypsicladia* or *M. indohii*. When cultured together with *M. alpina* or *M. reticulata*, spore germination could be seen, but sporulation was scarce. When using *Arthrobotrys* sp. and *Lecanicillium* sp. as possible host fungi, spore germination was never seen. These observations suggest that the mycoparasitism of *N. mortierellicola* is specific to certain species of *Mortierella*.

Sporangia lacking a columella are characteristic of species of *Mortierella* (*Mortierellales, Mortierellaceae*) and *Umbelopsis* (*Mucorales, Umbelopsidaceae*) (O'Donnell *et al.* 2001, Meyer & Gams 2003). The affinity of *Nothadelphia* to either of these genera cannot yet be assessed and must await molecular analysis, but the initially elongate "vesicle" is suggestive of a sporangium with a subsporangial septum of the second group.

Species of *Mortierella* can be hosts for several mycoparasites of the Zoopagalean fungi (*Piptocephalidaceae*). As *Nothadelphia* does not grow without a host on shrimp, brain-heart-infusion or malt-yeast extract agar media and does not kill its *Mortierella* host, its nutritional mode of parasitism is obviously biotrophic. Its parasitism has in common with species of *Piptocephalis* the production of minute haustoria. At the same time, the fungus induces the host *Mortierella* to form a dense mass of dichotomous or irregularly branched swollen hyphae, near a germinating spore of the parasite. Such 'gall'-like structures are known in the genus *Syncephalis*: on *Rhizopus stolonifer* by *S. californica* W.E. Hunter & E.E. Butler (1977); on *Mortierella alpina* by *S. parvula* Gruhn (in Gruhn & Petzold 1991). However, these two genera of the *Piptocephalidaceae* produce merosporangia; multispored spherical sporangia are not known in the *Zoopagales* (Benjamin 1979).

The chlamydospores of *Nothadelphia* might be parthenogenetic sexual spores. Thick-walled papillate structures are not rare for the oospores of the *Oomycota* or resting spores of the *Chytridiomycota*. In *Pythium* stalked zoosporangia are produced at the tip of mycelia, and several species are well-known mycoparasites of true Fungi (e.g. *Pythium oligandrum* Drechsler, *P. mycoparasiticum* Deacon *et al.* 1991).

Fig. 3A–U. *Nothadelphia mortierellicola.* A. Growth habit on natural substrate (bat dung) on agar plate (Sh3A). Sporangiophores radiating from dense hyphal mass with chlamydospores of *Mortierella hypsicladia.* B, C. Two-membered culture of *N. mortierellicola* with *M. hypsicladia* on agar plate (Sh3A); bundles of sporangiophores emerging from points of spore inoculation. D, E. Tips of sporangiophores. F. Sporangiospores. G, H. Swollen spherical sporangiospores, with haustoria prior to germination into vegetative hypha. I. Swollen sporangiospore with germinated vegetative hypha (arrowhead). J. Vesicles with vegetative hypha at bases, and sporangiophore stalk at the tips. K. One vesicle (Ve) with sporangiophore (Sp), connected with intercalary chlamydospore (Ch) by a vegetative hypha (Vh). L, M. Young vesicles, with vegetative hypha (arrowhead points to subvesicular septum). N. Sporangium. O. Mature sporangium containing sporangiospores. P. Young sporangium. Q. Strongly swollen sporangiospores with haustorium formation around vegetative hyphae of the host *Mortierella*. R. Young stage of chlamydospore. S, T. Mature chlamydospores (the same material with different focusing), with papillate ornamentation and hyphal segment remaining at the distal end. U. Mature chlamydospore stained by phloxine in KOH. Scale bars: A, B = 1 mm, C = 10 mm, D–F, G–I = 10 µm, J–K, L–P, Q = 20 µm, R–U = 10 µm.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The unique mycelial system of *Nothadelphia* also suggests a similarity with some kind of polycentric chytrids. Haustorial spores germinated into vegetative hyphae that produce vesicles at the distal end, from which many erect sporangiophores with wet sporangia are borne. A 'chytrid sp.' (Willoughby 1966), which was regarded as related to *Saccopodium* Sorokin, had stalked zoosporangiophores. Among the many mycoparasitic species of the Phylum *Chytridiomycota*, the genus *Caulochytrium* shows a haustorial biotrophism (Voss & Olive 1968, Voss 1969, Powell 1981) which superficially resembles that of *Piptocephalis* (Jeffries & Young 1994) and *Nothadelphia*.

However, both these phyla produce zoospores within the zoosporangia. The present fungus never produced flagellate spores even if cultured under water. If the present fungus belonged to either of these phyla, it should have lost flagellum formation secondarily, as an adaptation to the terrestrial environment. Until molecular and ultrastructural studies are completed, we prefer to regard this peculiar mycoparasite as an aplanosporic lower fungus incertae sedis in the Zygomycetes.

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