***Atractilina alinae* sp. nov. and *Neosporidesmium vietnamense* sp. nov. – two new synnematos hyphomycetes from Vietnam**

Vadim A. Mel'nik^{1*} & Uwe Braun²

¹Laboratory of the Systematics and Geography of Fungi, Komarov Botanical Institute, Russian Academy of Sciences, Professor Popov Street 2, St. Petersburg 197376, Russia

²Martin-Luther-Universität, Institut für Biologie, Bereich Geobotanik und Botanischer Garten, Herbarium, Neuwerk 21, 06099 Halle (Saale), Germany

Received 26 September 2013 / Accepted 21 October 2013 / Published 3 November 2013

Mel'nik, V.A. & Braun, U. 2013. *Atractilina alinae* sp. nov. and *Neosporidesmium vietnamense* sp. nov. – two new synnematos hyphomycetes from Vietnam. – Mycobiota 3: 1–9. doi: 10.12664/mycobiota.2013.03.01

Abstract. The new species *Atractilina alinae* and *Neosporidesmium vietnamense*, collected in Vietnam on fallen leaves of an unidentified tree and on bark of a twig of an unknown tree, respectively, are described, illustrated and discussed. *A. alinae* resembles the hyperparasitic *A. parasitica*, but differs in having quite distinct conidia. *Neosporidesmium vietnamense* is morphologically unique by its synnematos, indeterminate conidiomata, integrated, terminal, monoblastic, non-proliferating conidiogenous cells and obclavate, rostrate, euseptate conidia, but taxonomically rather complex with regard to its generic affinity.

Key words: *Ascomycota*, asexual morphs, new species, Southeast Asia, synnemata, Vietnam

Introduction

Numerous specimens of fungi have been collected within the scope of a research program of the Vietnam-Russian Tropical Research and Technological Centre. In a series of papers, Mel'nik (2011, 2012a, b) and Mel'nik et al. (2012, 2013) published results of examinations of several Vietnamese hyphomycetes, including numerous new records and some new species. Additional collections found on fallen leaves and on bark of a twig of unidentified trees turned out to be new species of *Atractilina* Dearn. & Barthol. and *Neosporidesmium*

*Corresponding author: e-mail: melnik@mail.ru

Mercado & J. Mena, respectively. Attempts to cultivate these fungi, in order to be able to carry out molecular sequence analyses, failed. However, these fungi are morphologically easily distinguishable from all other species of *Atractilina* and *Neosporidesmium* and are hence described as new species.

Materials and methods

Fresh samples collected in the course of field trips in Vietnam were dried at room temperature. The collections were later examined in distilled water and photographed using a Zeiss microscope, Stemi 2000CS, and Axio Imager A1 equipped with Nomarski differential interference contrast optics. The identification of the new species was accomplished through comparison with descriptions of other species hitherto assigned to *Atractilina* and *Neosporidesmium*.

Taxonomic treatment

Atractilina alinae Melnik & U. Braun, sp. nov.

Fig. 1

MycoBank, MB 805921

Atractilinae parasiticae similis, sed conidiis brevioribus, (20–)30–35(–40) μm, (3–)4–7-septatis, hyalinis vel dilute flavido-viridulis, levibus, non rostratis, apice non inflato, non hyperparasiticis.

Conidiomata synnematos on the natural substrate, solitary, scattered, dark brown to blackish. Synnemata determinate, up to 500 μm long, composed of a firm, brown, subcylindrical stalk, 10–25 μm wide, base often somewhat wider, up to 30 μm, and a rather compact, paler head, subglobose in outline, up to 80 μm diam., individual appressed filaments of the stalk 1.5–4 μm wide, pigmented, septate throughout, wall smooth, thin to slightly thickened, filaments apically slightly to moderately splaying out, occasionally branched, free part up to 45 μm long and 2.5–5 μm wide, pale to medium brown, tips often paler; conidiogenous cells integrated, terminal, up to 35 μm long, polyblastic, with a single or several distinct denticles, proliferation sympodial; denticles subcylindrical to usually conical, 1–2 × 0.8–1.5 μm, apex truncate, thin-walled. Conidia formed singly, broadly fusiform, ellipsoid, attenuated towards base and apex, (20–)30–35(–40) × (3–)4–7 μm, (2–)4–7-septate, septa thin and rather inconspicuous, subhyaline to faintly yellowish green, thin-walled, smooth or almost so, apex subobtuse to pointed, base obconically truncate, hila 1–1.5 μm wide, neither thickened nor darkened or only slightly darkened by being refractive.

Type on decaying leaves of an unidentified tree: Vietnam, Dong Nai Prov., Cat Tien National Park, Nam Cat Tien Sector, right bank of the river Dong Nai, polydominant monsoon lowland tropical forest, near the climatological tower, 11°26'25"N, 107°23'59"E, 22 Nov 2012, Yu. Novozhilov (**holotype**, HAL 2605 F; **isotype**, LE 263984).

Etymology: dedicated to Alina V. Alexandrova for her contribution to the knowledge of Vietnamese fungi.

Fig. 1. *Atractilina alinae*, micrographs (based on type material), A – synnemata, B, F, G – conidiogenous cells, C, D, E – conidia. Scale bars: A = 50 µm and B–G = 10 µm

Neosporidesmium vietnamense* Melnik & U. Braun, sp. nov.*Fig. 2**

MycoBank, MB 805922

Neosporidesmii micheliae similis, sed synnematibus longioribus, (380–)500–1430 µm, et latioribus, ad basim 40–45 µm, conidiophoris angustioribus, 2–2.5 µm latis et conidiis longioribus et latioribus, (75–)80–96(–110) × 11–13.5 µm, laevibus, verrucosis vel pagina inaequali, apice subhyalino.

Conidiomata synnematosus on the natural substrate, on bark, solitary, scattered, dark brown to blackish. Synnemata indeterminate, erect, straight, (380–)500–1430 µm high, stem somewhat decreasing in width from base to top, 40–45 µm wide at the base and 18–20 µm in the apical portion, composed of parallel, tightly appressed, dark brown, smooth, septate, thin-walled threads (conidiophores), 2–2.5 µm wide, without distinct head, diverging terminally and laterally, splaying out portions mainly consisting of terminal conidiogenous cells, fertile zone covering the upper quarter to third of the synnemata. Conidiogenous cells integrated, terminal, monoblastic, determinate, doliiform to lageniform, usually somewhat curved, 9–11 × 5–7.2 µm, wall dark brown, thin to somewhat thickened, smooth, conidiogenous locus at the truncate apex, 4–4.5 µm wide, unthickened, concolorous with the lateral wall. Conidia solitary, formation holoblastic, dry, obclavate, gradually tapering towards the apex, rostrate, base obconically truncate, (75–)80–96(–110) × 11–13.5 µm, 9–12-euseptate, not constricted at the septa, cells often with distinct lumen, pale brown, paler at the apex, subhyaline, wall thin to somewhat thickened, smooth to somewhat rough, surface at least not quite even, hila 3–4 µm wide, unthickened and not darkened.

Type on bark of a dry, thin branch of an unidentified tree: Vietnam, Dong Nai Prov., Cat Tien National Park, Nam Cat Tien Sector, Ma Da, near a small bridge close to Memorial of Defenders, monsoon tropical forest with prevalence of lianas and palms (*Calamus* spp.), 11°23'02"N, 107°03'40"E, 11 Jan 2011, leg. Yu. Novozhilov (**holotype**, DAOM 242720; isotype, LE 263986).

Discussion

Due to the formation of true synnemata, denticulate conidiogenous cells and pale pluriseptate conidia, the Vietnamese hyphomycete, collected on fallen leaves of an unidentified tree species, may easily be identified as member of *Atractilina* (Deighton & Pirozynski 1972; Ellis 1976; Seifert et al. 2011). Only a few species have been assigned to this genus, including some non-synnematous species which are probably not congeneric. Deighton & Pirozynski (1972) introduced the combination *Atractilina asterinae* (Hansf.) Deighton & Piroz. for a species hyperparasitic on sooty molds in Africa. This species is non-synnematous, i.e. it forms solitary conidiophores, and the conidia are distoseptate. Therefore, it is very doubtful if this species has been correctly assigned to *Atractilina*. *A. hymenaeae* Bat. & J.L. Bezerra (Batista et al. 1961), described from Brazil on *Hymenaea* sp., associated with *Asterina* and *Asteridiella* spp., is also non-synnematous and very probably not correctly placed in *Atractilina*. *A. alinae* roughly resembles *A. parasitica* (G. Winter) Deighton & Piroz. (Deighton & Pirozynski 1972; Ellis 1976), a hyperparasitic species

Fig. 2. *Neosporidesmium vietnamense*, micrographs (based on type material), A – synnemata, B–D – apex of synnema with conidiogenous cells (B and D in addition with conidia), E–F – conidia. Scale bars: A = 500 μ m, B–F = 10 μ m

widespread in tropical countries, but the latter species has quite distinct conidia which are longer, 35–50 µm, mostly 3-septate, often minutely verruculose and slightly pigmented with age, frequently with beak-like apex and a terminal small knob. The synnemata are up to 1.5 mm long and 40 µm wide. *A. calycini* Jana, A.K. Das & S.N. Ghosh (Jana et al. 2006), described from India on living leaves of *Neurocalyx calycinus*, is morphologically very close to *A. parasitica* and said to be distinguished by shorter synnemata, up to 900 µm, occasionally branched conidiophores wider near the apex, and smaller, always smooth conidia without terminal knob. The ecology of this species is unclear and not properly specified. In the description, *A. calycini* is classified to be hyperparasitic, but its mycelium is described to grow superficially on living leaves. Other fungi on the host on which this species might be developed as hyperparasite are not mentioned. *A. biseptata* R.F. Castañeda (Castañeda-Ruiz 1986) is a true synnematous *Atractilina* species, but differs from the Vietnamese fungus in having consistently 2-septate conidia.

The generic affinity of the new species tentatively assigned to the genus *Neosporidesmium* (Mercado & Mena 1988) is rather complex and needs to be discussed in detail. This fungus is well-characterized by forming synnematous indeterminate conidiomata with integrated, terminal, monoblastic, non-proliferating conidiogenous cells and obclavate, rostrate, euseptate conidia formed singly. Based on these traits, this fungus is easily referable to a complex of synnematous genera around *Morrisiella* Saikia & A.K. Sarbhoy (= *Hemisynnema* Subram.), *Neosporidesmium*, *Novozymia* W.P. Wu, *Podosporium* Schwein, as well as *Sporidesmina* Subram. & Bhat and may be considered a species of the *Sporidesmium* complex s. lat. (Wu & Zhuang 2005; Seifert et al. 2011). However, using the key to *Sporidesmium*-like genera in Wu & Zhuang (2005) and the general key to synnematous hyphomycete genera in Seifert et al. (2011), and the descriptions and illustrations in these works, no perfectly fitting genus has been found. Wu & Zhuang (2005) recognized *Morrisiella* as genus of its own, and *Hemisynnema* was reduced to synonym with this genus. Furthermore, they considered *Janetia synnematos* Sivan. & W.H. Hsieh a synonym of *Morrisiella indica* Saikia & A.K. Sarbhoy, the type species of *Morrisiella*. In contrast, Seifert et al. (2011) listed the latter genus as synonym of *Janetia* M.B. Ellis. The conidiogenous cells of *M. indica* and the new fungus from Vietnam are very similar, but *Morrisiella* differs in having laterally integrated, pleurogenous conidiogenous cells, and the conidia are distoseptate. The structure of the conidiogenous cells in *Novozymia* is also very similar, but they proliferate percurrently, leave distinct annellations, and the conidia are distoseptate. *Podosporium* is another superficially similar genus with euseptate conidia, but it can be readily ruled out due to its tetric conidiogenesis. *Sporidesmina malabarica* Subram. & Bhat, the type species of *Sporidesmina*, has unique synnemata with a carbonaceous structure, characteristically crowded conidiogenous cells, and the conidia are eu- and distoseptate and form a terminal globose mucilaginous appendage. Last but not least, *Neosporidesmium* has to be taken into consideration. This genus was introduced for a synnematous species with monoblastic conidiogenous cells, percurrent proliferation resulting in a succession of swollen, almost monilioid cells, and solitary pluridistoseptate conidia (Mercado & Mena 1988). Wu in Wu & Zhuang (2005) described *N. microsporum* W.P. Wu, which is morphologically close to the type species of *Neosporidesmium*, and added the new species *N. sinense* W.P. Wu, a species with non-proliferating conidiogenous cells,

leading to a significant widening of the generic circumscription of the latter genus. Additional species have been described in Ma et al. (2011), viz. the proliferating *N. antidesmatis* Jian Ma & X.G. Zhang (as “*antidesmae*”), and the two non-proliferating taxa *N. malloti* Jian Ma & X.G. Zhang and *N. xanthophylli* Jian Ma & X.G. Zhang. A second significant widening of the generic concept of *Neosporidesmium* has been made by the description of *N. micheliae* Y.D. Zhang & X.G. Zhang, a Chinese species characterized by having euseptate conidia (Zhang et al. 2011), which enables us to assign the new Vietnamese species to the latter genus. *N. micheliae* is quite distinct from *N. vietnamense* and easily distinguishable by having much shorter and narrower synnemata, up to 530 µm long and 20–30 µm wide below, wider conidiophores, 4.5–6.5 µm, as well as smooth, shorter and narrower conidia, 40–60 × 8.5–11 µm, with pale brown, non-rostrate apex. All other species of this genus differ in having distoseptate conidia. The only rational alternative to an inclusion in *Neosporidesmium* would be the introduction of a new genus for *Neosporidesmium*-like species distinguished by forming euseptate conidia, which would strictly follow the generic concept of Wu & Zhuang (2005), who accepted Subramanian’s (1992) splitting of the *Sporidesmium* Link s. lat. complex by keeping genera like *Sporidesmium* Link s. str. (euseptate conidia) and *Ellisembia* Subram. (distoseptate conidia) as separate taxa. However, the taxonomic relevance of conidial septation (eu- and distosepta) as well as the formation of mononematous versus synnematus formations of conidiophores within the *Sporidesmium* complex is quite unclear and not yet sufficiently phylogenetically proven. Shenoy et al. (2006) published results of comprehensive molecular sequence analyses of a wide range of taxa of the *Sporidesmium* complex suggesting that *Sporidesmium*, *Ellisembia* and other segregated genera are not monophyletic. Particular species of these genera are phylogenetically distributed in two major ascomycete classes, *Dothideomycetes* and *Sordariomycetes*. A single sequence referred to as *Neosporidesmium* sp. clustered in the *Dothideomycetes* adjacent to *Sporidesmium australiense* M.B. Ellis. Under these circumstances, it does not make any sense to add a further genus just based on a single morphological character.

The following key to species of *Neosporidesmium* is based on a key previously published by Ma et al. (2011), adapted and supplemented by inclusion of *N. micheliae* and *N. vietnamense*:

- 1 Conidia euseptate 2
- 1* Conidia distoseptate 3
- 2 Synnemata (380–)500–1430 µm long, 40–45 µm wide below, conidiophores 2–2.5 µm wide, conidia (75–)80–96(–110) × 11–13.5 µm, wall smooth to somewhat rough, surface at least not quite even, rostrate apex subhyaline *N. vietnamense*
- 2* Synnemata shorter, up to 530 µm long, and narrower, 20–30 µm wide below, conidiophores wider, 4.5–6.5 µm, conidia shorter and narrower, 40–60 × 8.5–11 µm, wall smooth, apex not distinctly rostrate and pale brown *N. micheliae*
- 3 Conidiogenous cells percurrently proliferating 4
- 3* Conidiogenous cells monoblastic, determinate, without any proliferation 6

- 4 Conidia distinctly rostrate *N. antidesmatis*
 4* Conidia non-rostrate 5
 5 Conidia 45–67 × 6–7 µm, 6–7-distoseptate *N. microsporum*
 5* Conidia much larger, 55–100 × 15–19 µm, 8–12-distoseptate *N. maestrense*
 6 Synnemata very broad, 80–110 µm at the base, conidia very long, 120–150 µm, with rather long rostrum *N. sinense*
 6* Synnemata much narrower, 20–55 µm at the base, conidia much shorter, about 20–50 µm, beak lacking or short 7
 7 Conidia 22.5–33.5 × 7–9 µm, 6–7-distoseptate, apex pale brown and not distinctly rostrate *N. malloti*
 7* Conidia somewhat longer and much broader, 33.5–51.5 × 12–14 µm, 6–10-distoseptate, with distinct, hyaline or subhyaline rostrum, 11–19 µm long *N. xanthophylli*

Acknowledgements. Sincere thanks are due to Yu. Novozhilov, who made the field collections in Vietnam and provided the material for further taxonomic treatment, and E. Popov for the final preparation and arrangement of the illustrations.

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