

***Entoloma piceinum*, a new lignicolous species of *Entolomataceae* (*Agaricales*) from the European Russia**

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Abstract. The new species *Entoloma piceinum* O. V. Morozova, Voronina et Arslanov of the section *Fernandae* (subgenus *Nolanea*), found at two sites of the European Russia (Saint Petersburg and Moscow Region), is described. As a member of the section *Fernandae* it is characterized by the presence of the intracellular pigment in form of dark brown clots combined with the dark encrusting pigment, and by the clampless hyphae. New species differs from the other members of this section by the combination of the subtomentose to distinctly squamulose pileus with inhabiting *Picea abies* wood. ITS sequences of both studied specimens have shown their conspecificity in spite of some differences in basidiomata morphology.

Keywords: agaricoid basidiomycetes, *Entolomataceae*, section *Fernandae*, new species, North Coast of the Neva Bay wildlife refuge, Zvenigorod Biological Station wildlife refuge.

Entoloma piceinum — новый деревообитающий вид семейства *Entolomataceae* (*Agaricales*) из европейской части России

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Резюме. Приводится описание нового вида *Entoloma piceinum* O. V. Morozova, Voronina et Arslanov из секции *Fernandae* (подрод *Nolanea*), обнаруженного в двух точках европейской части России (Санкт-Петербург и Московская обл.). Как представитель секции *Fernandae* вид характеризуется наличием внутриклеточного пигмента в форме темно-коричневых сгустков в сочетании с темным инкрустирующим пигментом, а также отсутствием пряжек на гифах. Новый вид отличается от других представителей этой секции слегка опушенной или отчетливо чешуйчатой поверхностью шляпки и обитанием на гнилой древесине ели (*Picea abies*). Сравнение последовательностей ITS образцов показало принад-

лежность обеих находок к одному виду, несмотря на имеющиеся морфологические отличия.

Ключевые слова: агарикоидные базидиомицеты, *Entolomataceae*, секция *Fernandae*, новый вид, заказник «Северное побережье Невской губы», Звенигородская биологическая станция.

Introduction

Section *Fernandae* Noordel. stands out within the subgenus *Nolanea* (Fr. : Fr.) Noordel. of the genus *Entoloma* (Fr. ex Rabenh.) P. Kumm. due to well differentiated pileipellis with two types of pigment (encrusting and intracellular in form of dark-coloured agglutinate clots or granules) and clampless hyphae (Noordeloos, 1980). According to the morphological species concept it includes seven species (Noordeloos, 1992, 2004). The preliminary molecular studies of subgenus *Nolanea* revealed that at least some species of the section *Fernandae* possibly belong to the different clades (Vila *et al.*, 2013). The circumscription of the section will be defined in the ongoing molecular studies.

All species of the section *Fernandae* described by now are terricolous. During the field surveys at summer — autumn 2013 two records of a lignicolous member of the section *Fernandae* have been made at Saint Petersburg and Moscow Region. In spite of the some morphological difference between specimens from two spatially distant points the comparison of the ITS sequences confirmed their conspecificity. It was assigned both by morphological and molecular analyses that the specimens belonged to a new, unknown species, described in this paper below.

Materials and methods

Both studied territories lie in the southern taiga. The first point was «North Coast of the Neva Bay» wildlife refuge, the territory under the administrative authority of Saint Petersburg. It is a strip of southern taiga vegetation along the coast of the Gulf of Finland. Coniferous as well as mixed forests with the participation of *Picea abies*, *Tilia cordata*, *Quercus robur*, *Acer platanoides*, *Betula pendula*, *Alnus glutinosa* are the main types of the vegetation (Atlas..., 2013). The record was made not far from the old-growth oak park. The second site was a territory of a wildlife refuge Moscow State University (MSU) Skadovsky Zvenigorod Biological Station (ZBS), western part of Moscow Region. The territory of ZBS represents vegetation typical of southern taiga with spruce (*Picea abies*) stands of different types. *Picea abies* is mixed with *Pinus sylvestris*, *Betula* spp. and some broad-leaved tree species at the most part of the area with isolated patches of nearly pure deciduous forests (Petrov, 1970).

The specimens were collected, documented and preserved using standard techniques. Macromorphological description was based on a study of the fresh and dried material as well as on analysis of the basidiomata photos. The dried material was examined using the standard light microscope techniques. Micromorphological structures were observed in squash preparations of small parts of the basidiome mounted in 5 % KOH and 1 % Congo Red in concentrated NH_4OH . Microscopic measurements and drawings were made with Zeiss AxioImager A1 light microscope. Basidiospore dimensions were made at the base of 20 spores observations. In spore descriptions Q is given for medium spore length to width ratios' value.

DNA was extracted from the dry material using Axy Prep Multisource Genomic DNA Miniprep Kit (Axygen Biosciences). The ribosomal ITS1-5.8S-ITS2 region was amplified by PCR with the fungal specific primers ITS1F and ITS4B (Gardes, Bruns, 1993). The PCR products were purified using AxyPrep PCR Cleanup Kit (Axygen Biosciences). Sequencing was performed with ABI model 3130 Genetic Analyzer (Applied Biosystems) using BigDyeTM Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems) with the same primers. The raw data were processed using Sequencing Analysis 5.3.1 (Applied Biosystems). Genetic distances between ITS sequences were estimated using MEGA 6.06 (Tamura et al., 2013).

Description

Entoloma piceinum O. V. Morozova, Voronina et Arslanov, sp. nov. (Fig. 1; Plate I).

Mycobank: MB809757

Diagnosis. *Entoloma piceinum* is a species of the section *Fernandae* (subgenus *Nolanea*), characterized by the presence of the intracellular pigment in form of dark brown clots combined with the dark encrusting pigment, and by the clampless hyphae. It differs from the other members of this section by the combination of subtomentose to distinctly squamulose pileus with the lignicolous habitat.

Holotype: Russia, St. Petersburg, Kurortniy District, vicinities of Lisiy Nos, «North Coast of the Neva Bay» wildlife refuge, 60°00'00.2" N, 30°01'52.8" E, on *Picea abies* stump in mixed (*Picea abies*, *Tilia cordata*, *Quercus robur*, *Betula* spp.) forest, 25.09.2013, leg. S. N. Arslanov, LE 254131. Genbank KM262035.

Pileus 12–23 mm broad, conical, broadly conical to convex with a small umbo, hygrophanous, translucently striate almost up to the centre, with a straight margin, dark grey-brown at the centre, paler towards margin, minutely squamulose all over with dark small squamules on the paler

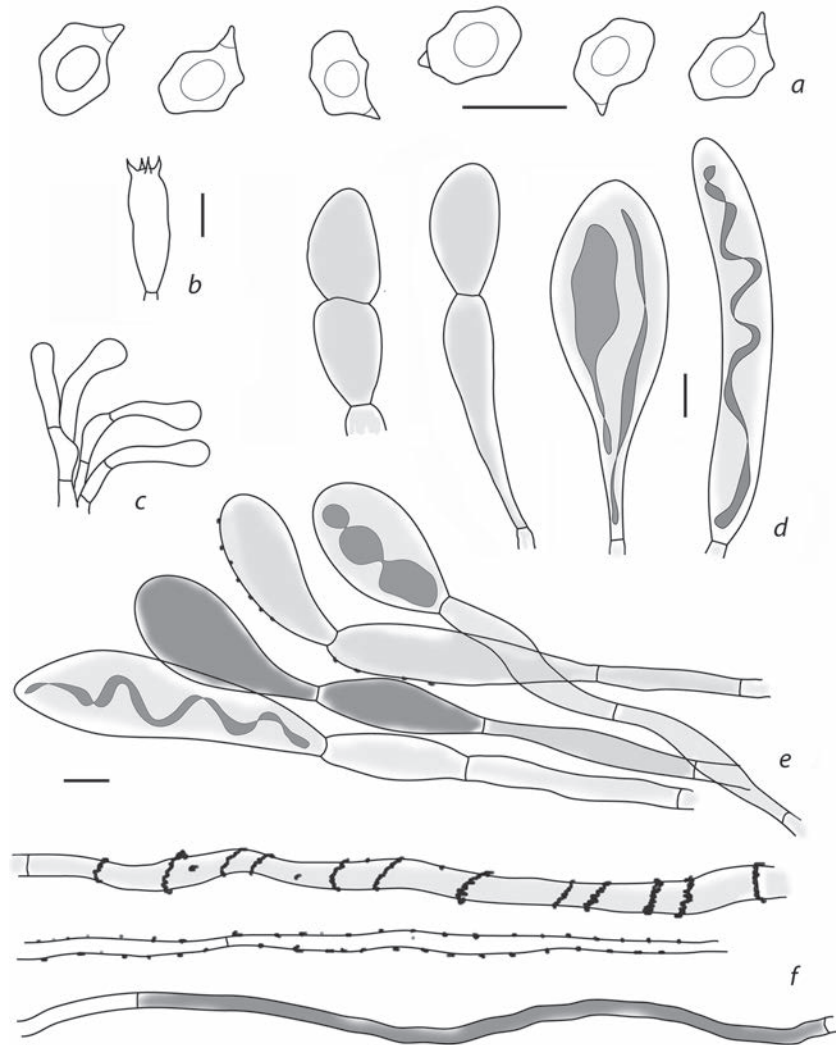


Fig. 1. *Entoloma piceinum* O. V. Morozova, Voronina et Arslanov (from LE 254131, holotype).

a — spores; *b* — basidium; *c* — caulocystidia; *d* — terminal cells of the pileipellis; *e* — pileipellis; *f* — hyphae of the subpellis. Scale bars: 10 μ m.

background, or felted. Lamellae moderately distant, adnate-emarginate or almost free, ventricose, whitish, becoming pink, with entire concolorous edge. Stipe 20–40 × 1–3 mm, cylindrical, smooth or slightly longitudinally fibrillose, fistulose, pale grey-brown, white tomentose at the base. Context concolorous with the surface. Smell indistinct, taste unknown.

Spores 8.5–11.0 × 5.6–6.7 μm, Q = 1.4–1.7, heterodiametrical, with 5–7 angles in side-view. Basidia 27.1–31.0 × 9.1–11.9 μm, 4-spored, narrowly clavate to subcylindrical, clampless. Lamellae edge fertile. Cheilocystidia absent. Pileipellis a cutis of cylindrical to slightly inflated hyphae 8–15 μm wide, with numerous ascending clavate, elongate to fusiform terminal elements 41.4–87.8 × 16.5–24.5 μm, containing intracellular pigment in form of dark brown clots, at times spirally arranged, sometimes also with brown encrusted walls. Subpellis composed of cylindrical cells 8.0–15.0 μm wide, with brown encrusted walls, often also with clots of intracellular pigment. Pileitrama regular, composed of cylindrical cells 6.0–13.0 μm wide, mixed with narrow (3.0–5.0 μm wide) hyphae, with brown encrusted walls, and diverticulate lactiferous hyphae. Stipitipellis a cutis of cylindrical hyphae 5–7 μm wide with pale intracellular pigment. Caulocystidia narrowly clavate to capitate, hyaline, 10.4–22.5 × 4.5–8.5 μm, in bundles. Clamp connections absent.

Habitat. On *Picea abies* stumps in mixed forests.

Additional specimen examined: **Russia**, Moscow Region, Odintsovskiy District, Zvenigorod town vicinities, MSU Skadovsky Zvenigorod Biological Station, quarter 1, 55°42'02" N, 36°43'20" E, on *Picea abies* stump with brown rot caused by polyporoid fungi in mixed forest (*Picea abies*, *Betula* spp., *Acer platanoides*, *Sorbus aucuparia* and *Padus avium*), 29.07.2013, leg. E. Yu. Voronina, LE 254132. Genbank: KM262036.

Discussion

The closest species to *Entoloma piceinum* is *E. fernandae* (Romagn.) Noordel. It is rather variable in its morphology and contains representatives with both glabrous as well as minutely squamulose pileus. The holotype of *E. piceinum* is characterized by the squamulose pileus. The pileus of the specimen from Moscow Region lacks distinct squamules, but the ascending terminal cells can be observed under light microscope. The dark brown agglutinate intracellular pigment prevails in the terminal cells of the holotype specimen. The encrusting pigment is more abundant in the specimen from Moscow Region. However both types of the pigment present in the both specimens. The molecular data confirmed that these

two specimens belong to the same species: the genetic distance between ITS sequences is 0.6 % base-pair difference.

Entoloma piceinum differs from *E. fernandae* by the larger spores and lignicolous habitat. Except habitat, the other species of the section *Fernandae* are distinguished from the new species: *Entoloma defibulatum* Arnolds et Noordel. by its rather weakly angled, smaller spores; *E. xanthocaulon* Arnolds et Noordel. by distinct yellow colour of basidiomes; *E. cuniculorum* Arnolds et Noordel. by differently shaped and larger spores; *E. kristiansenii* Noordel. by well differentiated cheilocystidia. *E. acidophilum* Arnolds et Noordel. and *E. argenteostriatum* Arnolds et Noordel. are characterized by the strongly striate lengthwise stipe surface.

One more species has been preliminary described as *Entoloma piceicola* Noordel. (ad interim) from the *Picea* trunk (Noordeloos, 1987). But hyphae of the pileipellis in this species contain pale intracellular pigment only, therefore this species was considered as close to *E. cetratum* (Fr. : Fr.) M. M. Moser and was placed within the section *Endochromonema* (Largent et Thiers) Noordel.

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References

- Atlas osobo okhranyaemykh prirodnikh territoriy Sankt-Peterburga* [Atlas of protected natural areas of St. Petersburg]. 2013. St. Petersburg: 176 p. (In Russ.).
- Gardes M., Bruns T. D. 1993. ITS primers with enhanced specificity for basidiomycetes — application to the identification of mycorrhizae and rusts. *Molec. Ecol.* 2: 113–118.
- Noordeloos M. E. 1980. *Entoloma* subgenus *Nolanea* in the Netherlands and adjacent regions with a reconnaissance of its remaining taxa in Europe. *Persoonia*. 10: 427–534.
- Noordeloos M. E. 1987. *Entoloma* (Agaricales) in Europe. Synopsis and keys to all species and a monograph of the subgenera *Trichopilus*, *Inocephalus*, *Alboleptonia*, *Leptonia*, *Paraleptonia*, and *Omphaliopsis*. *Beih. Nova Hewigia*. 91: 1–419.
- Noordeloos M. E. 1992. *Entoloma* s. l. *Fungi Europaei*. Vol. 5. Saronno: 1–760.
- Noordeloos M. E. 2004. *Entoloma* s. l. Supplemento. *Fungi Europaei*. Vol. 5a. Alassio: 761–1378.

- Petrov V. V. 1970. On original forest vegetation types at the territory of MSU Zvenigorod biostation. *Vestn. MGU. Ser. Biol., Pochv.* 3: 50–54. (In Russ.).
- Tamura K., Stecher G., Peterson D., Filipiński A., Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis. Version 6.0. *Molec. Biol. Evol.* 30: 2725–2729.
- Vila J., Carbó J., Caballero F., Català S., Llimona X., Noordeloos M. E. 2013. A first approach to the study of the genus *Entoloma* subgenus *Nolanea* sensu lato using molecular and morphological data. *Fungi non Delineati. LXVI (Studies on Entoloma)*: 3–62, 93–135 (iconography).

Литература

- [Atlas...] *Атлас особо охраняемых природных территорий Санкт-Петербурга*. 2013. Отв. ред. В. Н. Храмов, Т. В. Ковалева, Н. Ю. Нацваладзе. СПб.: 176 с.
- Gardes M., Bruns T. D. 1993. ITS primers with enhanced specificity for basidiomycetes — application to the identification of mycorrhizae and rusts. *Molec. Ecol.* 2: 113–118.
- Noordeloos M. E. 1980. *Entoloma* subgenus *Nolanea* in the Netherlands and adjacent regions with a reconnaissance of its remaining taxa in Europe. *Persoonia*. 10: 427–534.
- Noordeloos M. E. 1987. *Entoloma* (Agaricales) in Europe. Synopsis and keys to all species and a monograph of the subgenera *Trichopilus*, *Inocephalus*, *Alboleptonia*, *Leptonia*, *Paraleptonia*, and *Omphaliopsis*. *Beih. Nova Hewigia*. 91: 1–419.
- Noordeloos M. E. 1992. *Entoloma* s. l. *Fungi Europaei. Vol. 5*. Saronno: 1–760.
- Noordeloos M. E. 2004. *Entoloma* s. l. Supplemento. *Fungi Europaei. Vol. 5a*. Alassio: 761–1378.
- [Petrov] Петров В. В. 1970. О коренных типах леса Звенигородской биостанции МГУ. *Вестник МГУ. Сер. биол., почв.* 3: 50–54.
- Tamura K., Stecher G., Peterson D., Filipiński A., Kumar S. 2013. MEGA6: Molecular Evolutionary Genetics Analysis. Version 6.0. *Molec. Biol. Evol.* 30: 2725–2729.
- Vila J., Carbó J., Caballero F., Català S., Llimona X., Noordeloos M. E. 2013. A first approach to the study of the genus *Entoloma* subgenus *Nolanea* sensu lato using molecular and morphological data. *Fungi non Delineati. LXVI (Studies on Entoloma)*: 3–62, 93–135 (iconography).



Plate I. *Entoloma piceinum* O. V. Morozova, Voronina et Arslanov.
1 — basidiomata (LE 254131, holotype); 2 — basidioma (LE 254132), photo by V. P. Prokhorov; 3 — terminal cells of the pileipellis (from LE 254131, holotype). Scale bars: 1, 2 — 1 cm; 3 — 10 μ m.