New records of two pyrophilous ascomycetes from Siberia: *Pyropyxis rubra* and *Rhodotarzetta rosea*

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Abstract: The paper reports the first findings of *Pyropyxis rubra* and *Rhodotarzetta rosea* from Asian Russia (West Siberia). The ecological conditions, i.e. vegetation, substrates and phenology of both species are described and compared with earlier publications. Also the morphological characteristics are described and depicted by photographic images and the differences between these quite similar species are highlighted. *P. rubra* has been cultivated *in vitro*. The paper provides descriptions of the anamorphic stage and culture growth on different media.
Keywords: Ascomycota, ecology, post-fire successions, *Pyronemataceae*, pyrophilous fungi, West Siberia.

НОВЫЕ НАХОДКИ ДВУХ ВИДОВ ПИРОФИЛЬНЫХ АСКОМИЦЕТОВ *PYROPYXIS RUBRA* И *RHODOTARZETTA ROSEA* В СИБИРИ

Пиропиксис красная (*Pyropyxis rubra*) – редкий вид, связанный с пирогенными сообществами, где он развивается в первые годы после пожара на почве как сапротроф или факультативный паразит. Ареал вида был изначально ограничен Северо-американским континентом, однако позже появились находки в Швеции и в европейской части России (Мордовский заповедник). Нами отмечено новое местонахождение пиропиксиса в Западной Сибири в окрестностях г. Ханты-Мансийска. Родотарцетта розовая (*Rhodotarzetta rosea*) отмечалась ранее в Европе и Северной Америке, а также на Дальнем Востоке России и также является пирофильным видом. Нами отмечена первая находка вида на территории Западной Сибири в окр. Юганского заповедника. Виды имеют схожий внешний облик и сложно различаются без подробного ознакомления.

В статье мы провели описание экологических условий произрастания, фенологии, морфологического строения двух видов в их сравнении, а также условий культивирования *P. rubra* на искусственных средах.

Introduction

Pyropyxis rubra (Peck) Egger is a pyrophilous species reported from post-fire habitats where it grows as a saprophyte and colonizes the seedlings of trees (Egger & Padden, 1986a, 1986b). Originally only known from North America, it was later recorded in Sweden (Eichwald, 1999) and recently in Russia (Bolshakov & Ivoylov, 2012). *Rhodotarzetta rosea* (Rea) Dissing & Sivertsen which is confusingly similar to *Pyropyxis rubra*, is known from a comparatively larger number of records from Europe, North America and the Far East of Russia. It is probably also a saprophytic pyrophilous species.

The scope of the present publication is to describe new records of both species from West Siberia along with morphological, ecological and — in the case of *P. rubra* — cultural characteristics.

Material and methods

Specimens were collected by random routes in different forest communities including post-fire locations. We did not attempt to assess the occurrence and abundance of the species.

Voucher specimens were collected in the field according to the standard scheme (Lodge *et al.*, 2004). Macroscopic pictures were taken using a Canon EOS 60D *in situ* and with a stereo microscope with a mounted camera.

Apothecia were examined microscopically from material rehydrated in tap water. Lugol's solution was applied for testing the iodine reaction. The preparations were dyed with Congo Red to improve the view of the structures of excipulum. Anamorphic stages from culture were examined in tap water in the vital stage. A Zeiss Axiostar microscope with an Achromat 40/0.65 objective (dry) and an Achromat 100/1.25 Oil immersion objective was used for examination. Micrographs were made with an AxioCam ERc5s digital camera.

Cultures were obtained from dried apothecia. Small parts of hymenium were placed onto agar medium thus obtaining polysperm cultures. The cultures were grown on three media: 1) potato dextrose agar (PDA): potatoes (300 g), glucose (10 g), agar (20 g), tap water (1000 g), pH = 7; 2) water agar (WA): agar (20 g), tap water (1000 g), pH = 7; 3) PDA with addition of 1/2 spoon of aspen-wood ash, pH = 8. Five replicates were made of each media.

In addition to the cultures on agar media, the mycelium was inoculated on three types of sterile substrates and grown in jars for two months. This was done to induce the teleomorphic stage of the species by growing in culture. Three substrates were composed as follows: 1) sand (500 g) + bran (50 g) + charcoal (½ spoon) mixture; 2) grain (300 g) + charcoal (½ spoon) mixture; 3) garden soil (300 g) + ash and charcoal (½ spoon) mixture.

Results

Morphological description

Pyropyxis rubra (Peck) Egger, *Can. J. Bot.*, 62 (4): 705 (1984).
Basionym: *Peziza rubra* Peck, *Ann. Rep. New York State Mus. Nat. Hist.*, 24: 95 (1872).

Apothecia first hemispherical, then urnulate to cupulate, sessile, up to 2 cm in diameter, 1 cm high, pink with paler outer surface and more intensively coloured hymenium, outer surface pubescent from short hairs (lens), becoming glabrescent when old, hymenial surface smooth, pubescent margin of the apothecium extending beyond the hymenium for 0.1 mm. The fruitbodies grow scattered to densely packed, forming bright beds covering the soil surface mixed with ash, charred wood and forest litter.

Ectal excipulum 0.7 (at base) to 0.2 (at margin) mm thick, consisting of *textura globulosa* of thick-walled hyaline cells (10–15 µm), cells of outer layer forming scarce chains at flanks and dense moniliform brownish hairs 50–80 µm long at margin. **Medullary excipulum** of tightly packed *textura intricata*, cell walls thickened but less than in the ectal excipulum. **Subhymenium** composed of intricate hyphae 3–5 µm broad arising from the layer of enlarged globose cells beneath. **Asci** cylindrical, gradually enlarged to upper part, †170–195 × 13–16 µm, with croziers, operculate, inamyloid. **Paraphyses** filiform, same length as asci, scarcely branched, 2–3 µm broad, septate, some filled with refractive hyaline vacuolar content in dead state. **Ascospores** ellipsoid, with several small to medium guttules when immature, but eguttulate at maturity, †14.2–17.4 × 7.7–9.8 µm, mean: 15.6 × 8.8 µm (n = 20).

Mycelium from agar colonies 2–10 µm broad, hyaline to brownish, septate, smooth to densely warted in old cultures; catenulate hyphae 7–9 µm broad, septate with constrictions, producing dense sclerotial tissue of thick-walled cells of *textura globulosa*; conidiophores up to 100 µm long, 3–5 µm broad, hyaline, septate, terminal fertile cells dichotomously branched and inflated up to 9 µm, producing abundant conidia on short denticles; conidia subglobose, obovate, elliptical, clavate to subcylindrical, with short denticles,

Fig. 1. ***Pyropyxis rubra*** – macroscopical features: A. Densely packed apothecia forming bright beds covering soil surface, B. Photograph of apothecia in studio showing size and shape (bar = 1 cm), C. Median section through the apothecium, photograph under the lens.

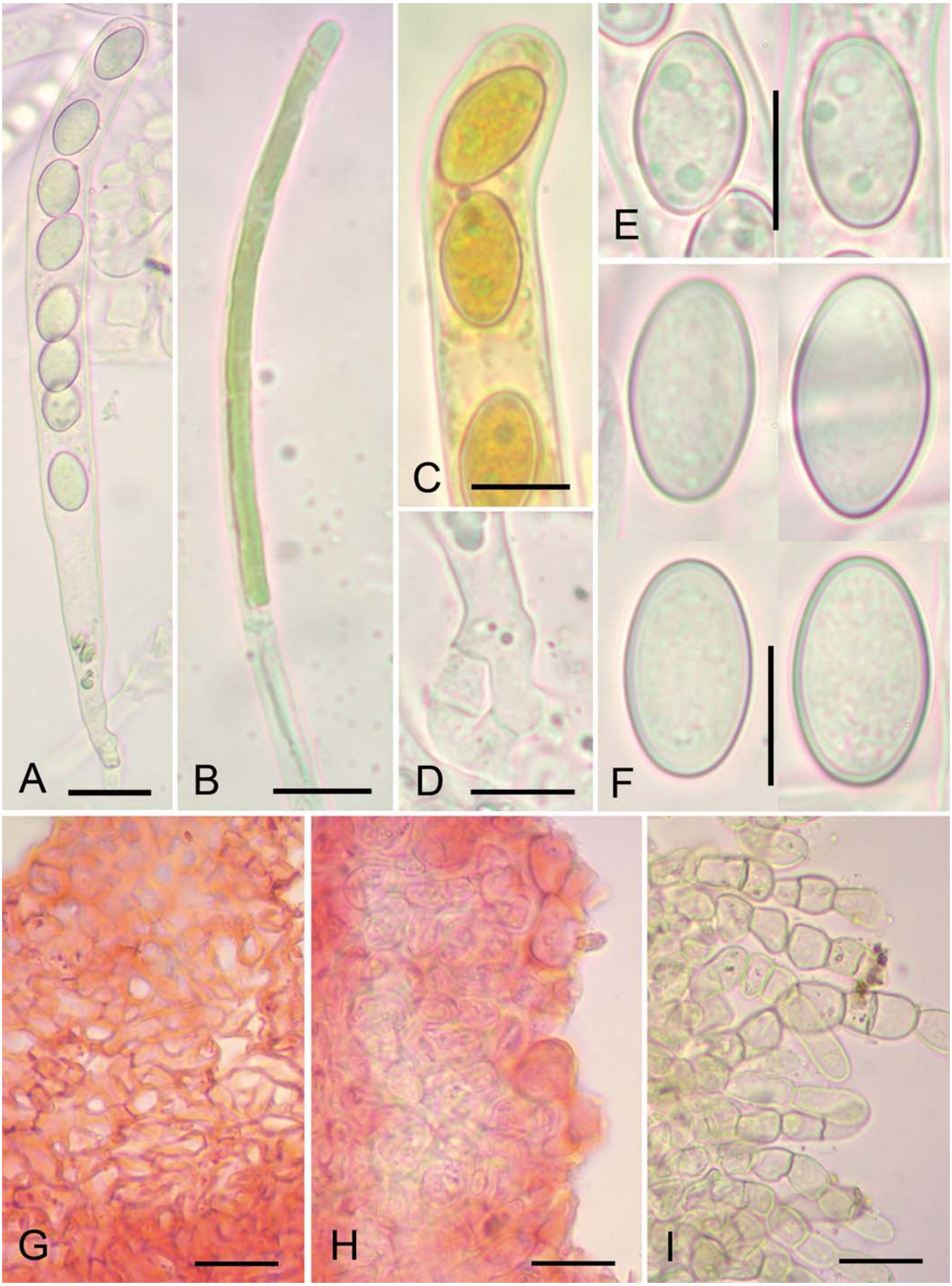

Fig. 2. *Pyropyxis rubra* – microscopic features: A. Ascus (dead state), B. Paraphysis (dead state) with vacuolar content, C. Ascus apical apparatus in IKI, D. Basal part of ascus, E. Immature spores containing small guttules, F. Mature spores lacking oil content, G. Medullar excipulum of *textura intricata* (CR), H. Ectal excipulum of *textura globulosa* made of thick-walled cells, I. Moniliform hairs of ectal excipulum. All structures examined from exsiccata (rehydrated in KOH). Scale bars in A, G, H, I = 20 µm and B, C, D, E, F = 10 µm.

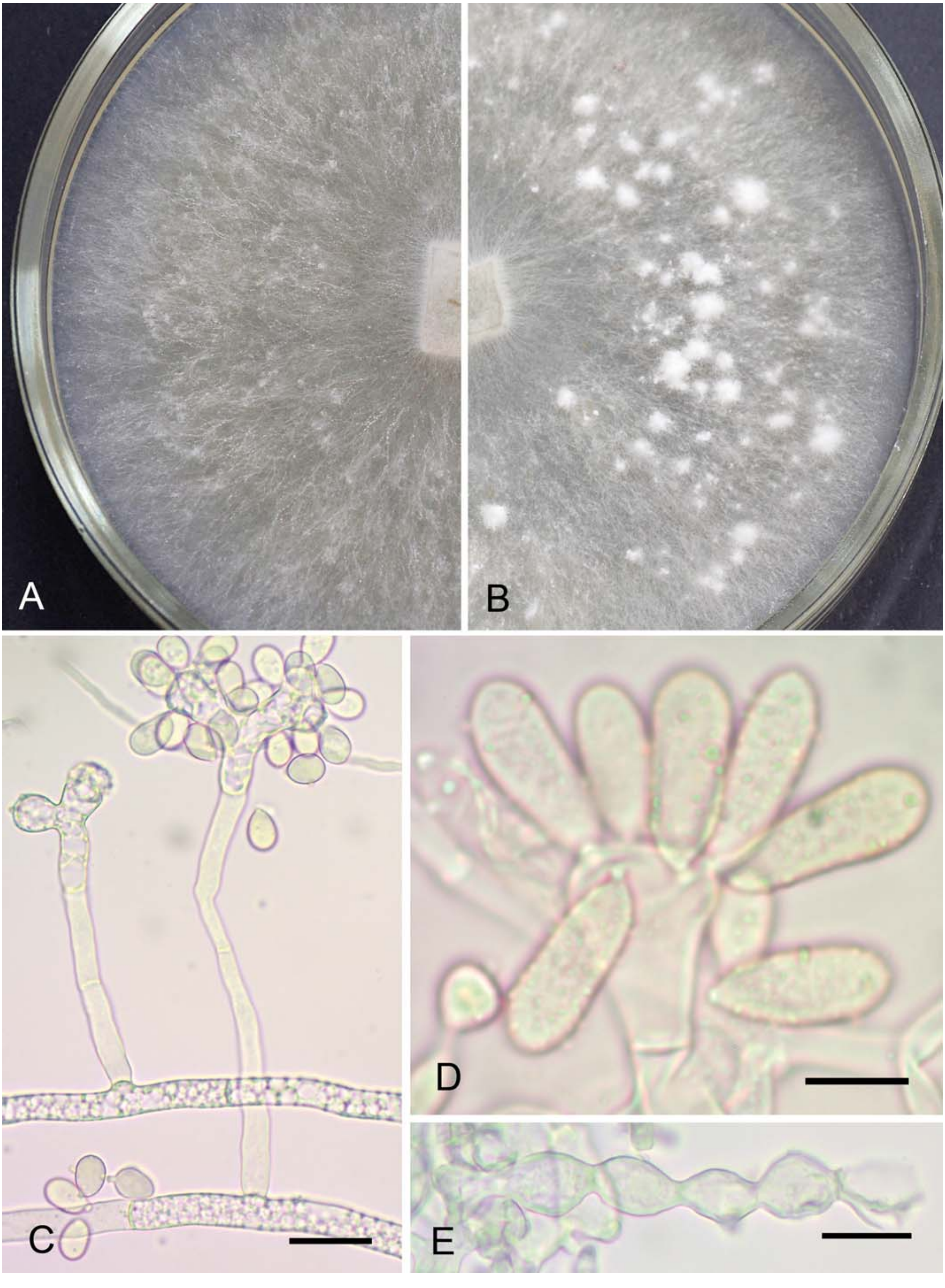

Fig. 3. ***Pyropyxis rubra*** – culture characteristics: A. Colonia view (conidial stage) on PDA, one week after inoculation, B. Same culture three weeks after inoculation with cottony clumps of catenuate hyphae, C. Conidiophores with dichotomously branched upper part producing conidia, D. Conidia, E. Catenuate hyphum with thick walls from sclerotium at later stages of culture development. All structures in vital state. Scale bars in D = 10 µm and C, E = 20 µm.

light brownish when mature, first smooth becoming more or less echinulate in old cultures with thin spines up to 1.5 µm high, *10–28 × 6–9 µm, mean: 16.5 × 8 µm (n = 30).

Studied collections: 1. Russia, Khanty-Mansiysk Autonomous Okrug, Khanty-Mansiysk vicinities, Mukhrino field station of the Yugra State University, 60.90019°N, 68.73659°E, 16 Jun. 2011, *leg*. T. Bulyonkova & N. Filippova, *det*. N. Filippova, Fungarium of the Yugra State University (YSU-F-03353). This finding was photographed *in situ*, collected and preserved as a dry specimen, studied microscopically, but after that the collection was lost and not available for further study. 2. Russia, same location, 19 Jun. 2012, *leg*. N. Filippova, *det*. U. Lindemann, Fungarium of the Yugra State University (YSU-F-03585) and private herbarium of Uwe Lindemann (U.L. 180-15). This collection was made in the same location, two years after fire. The specimens were photographed *in situ* and in laboratory under the lens. This specimen was used for cultivation and its anamorphic stages grown on different media are stored as dry cultures.

Rhodotarzetta rosea (Rea) Dissing & Sivertsen, *Mycotaxon*, 16 (2): 456 (1983).

Basionym: *Pustularia rosea* Rea, *Trans. Worcestershire Nat. Club*, 8: 20 (1924).

Synonym: *Tarzetta rosea* (Rea) Dennis, *Brit. Ascom*.: 30 (1978).

Apothecia sessile, cupulate with slightly involute sinuous margin, partially embedded in substrate, uniformly bright pink with a slight lilac tinge at center, 3–7 mm in diameter; external surface smooth, concolorous.

Ectal excipulum composed of ramose, tangled, partially inflated elements, often in chains, 9–24 (mean 15, n = 22) µm wide and 19.7–60 (mean 35.1, n=22) µm long, ventricose-cylindrical, utriform, fusiform, clavate or irregular-shaped, some with walls up to 2.5 µm thick, cemented by a thin external layer of gelatinous material; hairs not observed; apothecium attached to the substrate via tangled, ramose filamentous hyphae 3.0–7.7 (mean 4.9, n = 20) µm wide. **Medullary excipulum** as *textura globulosa*, 200–700 µm thick of tightly packed thin-walled inflated hyaline elements 13–40 (mean 25, n = 20) µm wide. **Subhymenium** a very tightly packed (preventing examination of individual structures) *textura intricata* 50–100 µm thick of thin-walled filamentous hyphae, appearing reddish en masse. **Asci** cylindrical, slightly tapering towards the base, †175–200 × 11–15 µm, with croziers, operculate, inamyloid. **Paraphyses** filiform, same length as asci, occasionally branched in the lower third, enlarged in upper part to 4–6 µm wide, with orange vacuolar pigment (in the rehydrated exsiccatum). **Ascospores** ellipsoid, smooth, hyaline, with several guttules when immature, ripe with two guttules or one large central guttule, †14.7–17.7 × 7.6–8.7 µm, mean 16.1 × 8.1 µm (n = 20).

Studied collections: Russia, Khanty-Mansiysk Autonomous Okrug, Surgutskiy rayon, Ugut vicinities, in Leikovskiy Bor, 60.35469°N, 74.01040°E, 25 Jul. 2015, *leg*. and *det*. T. Bulyonkova, YSU-F-06578 and in the private collection of Tatiana Bulyonkova (T.B. #3530).

Culture growth of *Pyropyxis rubra*

PDA: Colonies fast growing, reaching 9 cm diameter in 3 days, greyish, with brownish conidial mass, with floccose overgrowth forming 2-3 indistinct radial zones, later forming abundant white cottony clumps throughout the colony. Conidia begin to form after 2–3 days. At later stages of colony development the white cottony clumps become dense warts and finally form sclerotia, they develop intensively during the colony die-off.

PDA with ash: Similar characteristics.

WA: Similar characteristics, but colonies much thinner, mycelium mostly submerged, slow growing and conidia barely forming.

Inoculation of three types of substrates (sand-bran-charcoal, grain-charcoal, and garden earth-charcoal) produced extensive growth with formation of conidia and sclerotia. No apothecia formed by the end of cultivation period (2 months). The most intensive colonization was observed in sand-bran-charcoal, while earth-charcoal supported the weakest growth.

Ecological conditions

The first record of *Pyropyxis rubra* was made in the vicinities of the Mukhrino field station of the Yugra State University in June 2011. An abundant fruiting of the species was discovered on a raised bank of the Mukhrina river, in a periodically flooded birch-aspen forest with admixture of conifers and shrubs, one year after fire. Apothecia were abundant on open soil covered by ash and charred litter in an area of 50 m^2, especially abundant fruiting was found on a slope of the Mukhrina river bank in a well-lit location. The fire had damaged the shrub and herbaceous layers approximately a year earlier (July 2010) but did not disturb the tree layer on the area of 300 m^2. Traces of charred debris and ash covering the soil were still present in June 2011. Besides the occurrence of *P. rubra*, the site was inhabited by other pyrophilous species: *Pholiota highlandensis* (Peck) Singer, *Coprinellus xanthothrix* (Romagn.) Vilgalys, Hopple & Jacq. Johnson, *C. domesticus* (Bolton) Vilgalys, Hopple & Jacq. Johnson, *Psathyrella pennata* (Fr.) A. Pearson & Dennis, *Anthracobia* sp., *Daldinia loculata* (Lév.) Sacc., *Morchella tomentosa* M. Kuo, *Peziza tenacella* W. Phillips, and *Plicaria endocarpoides* (Berk.) Rifai. The second record of *P. rubra* comes from the same location visited one year later (June 2012), where it was fruiting much less abundantly compared to the first year. The traces of fire were almost gone and shifts in other fungal community composition also occurred.

Rhodotarzetta rosea was recorded at a significant distance in the vicinities of the Yuganskiy Nature Reserve. The fruitbodies grew in an area recovering after a low-intensity fire of 2013 in a *Cladonia* – Scots Pine forest. Fruitbodies grew on a well-lit southern microslope above a marshy creek flowing through a boggy former oxbow lake, in a small group covering an area of about 10 × 10 cm on charred sandy soil, among fresh shoots of *Funaria hygrometrica*.

Discussion

Pyropyxis rubra

The known distribution of *P. rubra* was originally restricted to North America where it was frequently encountered in eastern Ontario and also recorded in New York (Highland) and British Columbia (Egger, 1984). The first European collection was reported in 1993 from Sweden (Eichwald, 1999). Two additional collections from Sweden in 2000 were reported in a short note of Eichwald (2002). A search of the Global Biodiversity Information Facility (GBIF) database yields 16 records of *P. rubra*, all from Sweden. They include the three reports mentioned above and 13 additional collections made in 2015 in the vicinities of Fagersta (Sweden). The first Russian collection of *P. rubra* was reported from Mordovia (Bolshakov & Ivoylov, 2012). Apothecia were collected on burned soil in a Scots Pine forest (approximate collection coordinates: 54.7823°N, 43.1833°E). The next collection came from Novgorod oblast' where the species was found by S. Arslanov on burnt soil in a post-fire coniferous forest (Eugene Popov, information based on the Botanical Institute of V.L. Komarov herbarium (LE) database). Our collections are significantly more eastern (about 2000 km) in relation to the previous Russian reports and located in the Asian part of Russia. Our collection is currently the northernmost report (61°N) of the species.

All known collections of *P. rubra* are related to post-fire communities, and thus the species is considered as a pyrophilous species (Dix & Webster, 1995). It has been registered mostly in post-fire deciduous and coniferous forests rather than open grassland landscapes. In relation to post-fire succession, *P. rubra* seems to belong to the group of species fruiting soon after burning (Dix & Webster,

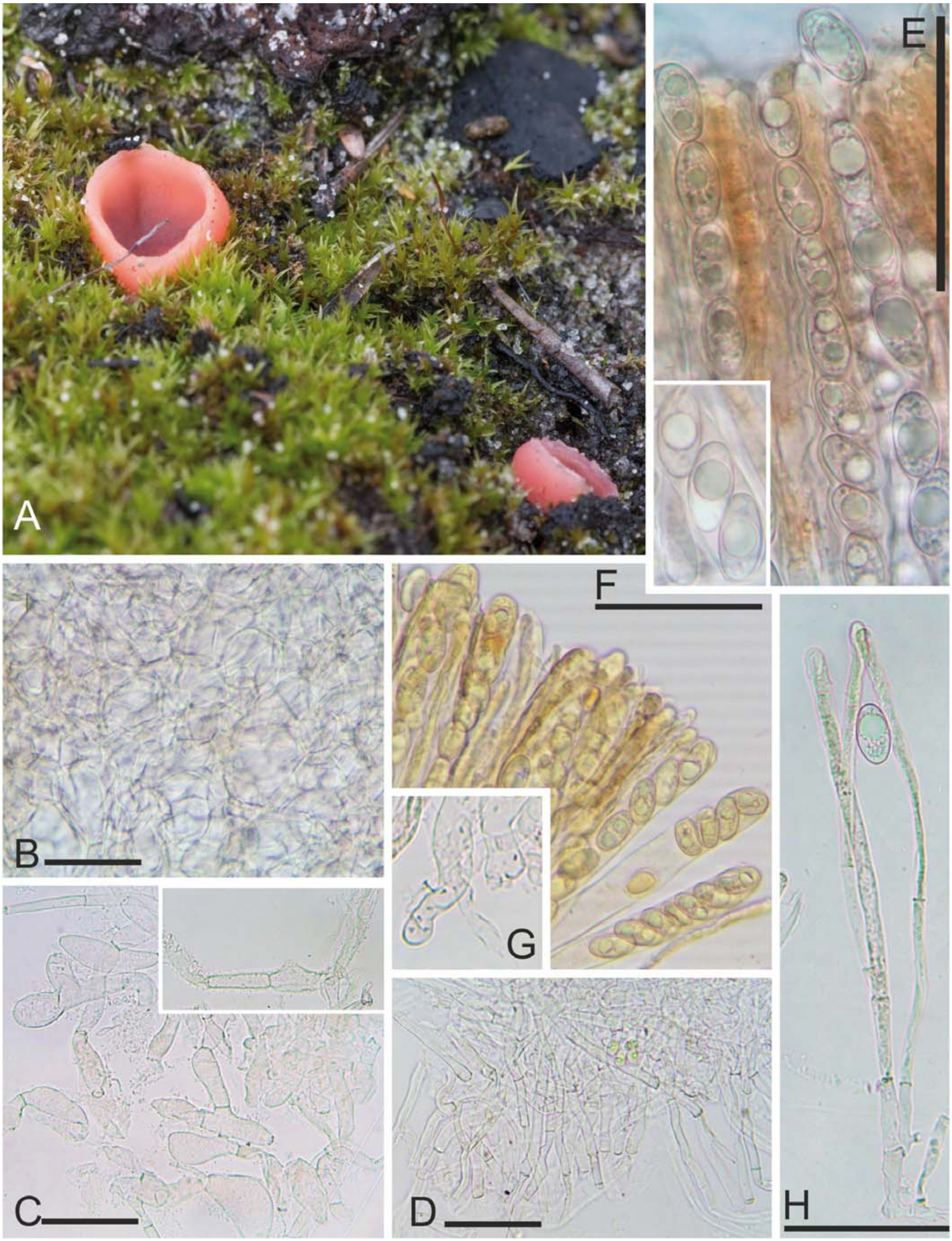

Fig. 4. *Rhodotarzetta rosea* – macroscopical and microscopical features: A. Apothecia *in situ*, B. Medullar excipulum of *textura globulosa*, C. Ectal excipulum, partially inflated hyphae in an intercellular matrix, D. Filamentous hyphae at the bottom of apothecium, E. Hymenium and ascospores, F. Ascus tips and paraphyses in Melzer's reagent, G. Crozier at ascus base, H. Paraphyses. All structures examined from the rehydrated exsiccate, except of F, which was rehydrated in 3% NaOH. Scale bars = 50 µm.

1995): it was frequently collected in a one-year-old fire by Egger (1984), it was found in Sweden one year after fire (Eichwald, 1999), and our observations registered a strong decline in abundance on a third year after fire (no apothecia were found in the site in the following years). In spite of the fact that many fungal species of post-fire successions are saprotrophic, *P. rubra* is considered to be facultatively pathogenic. Under experimental conditions it caused pathogenic infection of germinants and seedlings of *Pinus contorta* (Egger & Paden, 1986a, 1986b). However, the enzymes of pectinase, cellulase and phenol oxidase were also registered (Egger, 1986). Therefore the ability of the species to live as a saprobe can not be excluded. In supporting this observation, its anamorphic stage easily grows in culture as a saprobe on simple media.

We successfully cultivated *P. rubra* but so far have managed to grow only its anamorphic stage. The anamorph is undemanding and grew well on simple media without additives. More intensive growth was observed during cultivation on rich media (PDA) compared to water agar. Conidial stages did not require addition of ash to the media (pH 8) for growth. We failed to obtain the teleomorphic sporulation in culture using different types of media and substrates.

The morphological characteristics of the teleomorphic and anamorphic stages of our collections coincide well with earlier descriptions from Canada (Egger, 1984) and Sweden (Eichwald, 1999). The characteristic of culture growth was similar to descriptions in Egger (1984) except of the conidia which in our cultures were formed abundantly without ultraviolet light treatment.

Rhodotarzetta rosea

R. rosea was originally described from Britain where it is presently known from many records in the Fungal Record Database of Britain and Ireland. The species was also registered in other countries of Northern and Central Europe: in Norway (Dissing & Sivertsen, 1983; Hansen & Knudsen, 2000; Brandrud, 2010), Finland (Korhonen, 2009), Denmark (Dissing & Sivertsen, 1983; Hansen & Knudsen, 2000), The Netherlands (Maas Geesteranus, 1967; Dissing & Sivertsen, 1983), Slovakia (Baranovič, 2016), Lithuania (Kutorga, 1986), Switzerland (Jakob & Senn-Irlet, 2015), Austria, Germany, and Estonia (data of GBIF). The species is said to be widely distributed in North America (Beug *et al.*, 2014). In Russia *R. rosea* was known originally from several registrations in the Far East (Bogacheva, 2005, 2009)[1]. Recently, new records from Central Russia in the vicinities of Saint-Petersburg and Moscow were reported on a Russian mycological Internet forum (Eugene Popov, personal communication).

The association of *R. rosea* with burned areas is supported by all publications mentioned. Nevertheless, the trophic status of the species is unclear. It could be saprophytic, as well as bryosymbiotic (similar to other species of *Octospora* lineage); an ectomycorrhizal status is also possible in regard to the phylogenetically close genus *Rhodoscypha* (Hansen *et al*., 2013). *R. rosea* was collected in both coniferous and deciduous (oak) forests (Brandrud, 2010). In our records, there is a remarkable similarity of habitat preferences between *R. rosea* and *P. rubra*: both species grow at well-lit burnt sites on slopes with southern exposure in proximity of flowing water. That could explain their apparent rarity (see below "Conservation"). Whether they are or not indeed stenotopic will remain an open question until more finds are reported.

Dennis (1968) and Dissing & Sivertsen (1983) provide a detailed morphological description of *R. rosea*; macro-photographs are provided by several publications: Baranovič (2016), Beug *et al.* (2014), Korhonen (2009), Petersen (2016), Storey (*in* Van Vooren, 2015), Rubio (1997). In comparison to these, our collection has smaller apothecia (3–7 mm) while the size could reach up to 2 cm in other descriptions. The outer surface of our apothecia is naked and waxy only with minute cottony fragments, but in the pictures of J.H. Petersen and M. Storey it is covered by loose white mycelium. This discrepancy could be explained by differences in age (our specimens were not very old) or moisture conditions. The structure of the excipulum in our collection is two-layered (featuring a medullar excipulum made up of a *textura globulosa*) while it was described and pictured by Dissing & Sivertsen (1983) as a uniform *textura intricata*. We cannot explain this difference, but probably it also relates to the age of examined apothecia. The spore size of *R. rosea* was: 17–20 × 9–11 µm in Dennis (1978), 16.5–17.9–19.8 × 7.6–8.7–9.9 µm in Dissing & Sivertsen (1983), 15–17 × 9 µm in Rea (1924) — but 16–20 × 7.5–9.5(–11) µm in Yao & Spooner (2003), measured from the same type collection. Compared with this range, our spore measurements are amongst the smallest but fit within the total range.

Comparison of *Pyropyxis rubra* and *Rhodotarzetta rosea*

The two species are quite similar to each other, but several morphological differences help to distinguish them reliably. The outer surface of *R. rosea* is glabrous-waxy or covered by a white mycelial layer in older specimens while it is finely downy from short dense hairs in *P. rubra* (more apparent in younger specimens and at the margin); the outer surface of *P. rubra* is lighter than the hymenium while in *R. rosea* both are concolorous. Furthermore, the ascospores in *R. rosea* are characterized by two large guttules which remain also in the mature state; on the contrary, the ascospores of *P. rubra* contain two guttules or several small guttules when immature but they are eguttulate when mature. The shape of the ascospores is also different: the ascospores of *P. rubra* are more fusoid (with relatively acute ends) while in *R. rosea* they are more obtuse. The two species are also reliably distinguishable by the structure of the ectoexcipulum which is a thick-walled *textura globulosa* in *P. rubra* and a *textura intricata* of slightly inflated and thick-walled hyphae cemented by a gelatinous substance and loose filamentous aerial hyphae in *R. rosea*.

Another species with a quite similar morphology is *Rhodoscypha ovilla* (Peck) Dissing & Sivertsen, but there are considerable differences in the shape and size of the ascospores. Also *R. ovilla* is not pyrophilous, but grows on soil and forest litter (Dissing & Sivertsen, 1983).

Conservation

The reduction of natural habitats (pristine forests and natural fires) creates the threat of extinction of closely associated pyrophilous species. For this reason, *R. rosea* is included in conservation lists by several countries. It is listed as a Near Threatened species in Norway (Kålås *et al.*, 2010) and also recorded in different protection status for Denmark, Finland, Germany and The Netherlands (in the Compiled European fungal Red List data base) (Otto, 2011). *P. rubra* is listed in the Red List of Finland (Otto, 2011) and is recommended for the Red List status in Mordovia Republic, Russia (Bolshakov & Ivoylov, 2012b). We do not have enough information on the abundance of the two species in the Khanty-Mansiysk Autonomous Okrug to draw conclusions regarding their conservation status. But it seems unlikely that their habitats in the region will decline in the near future. Both species appear to be rare compared to other pyrophilous species and additional study of their ecology and habitat would be very valuable.

Acknowledgements

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[1] Bogacheva (2009) describes the range of *R. rosea* in the Far East of Russia without detailed description of collection sites: in Daurskiy (54N 123E), Okhotskiy (61N 145E), Ussuriyskiy (47N 135E) and North-Sakhalinskiy (53N 143E) floristic regions. The same author (Bogacheva, 2005) describes a collection from Sakhalin island in Bauri river valley (51N 143E), where it was collected on bare soil (VLAD-2021, VLAD-2030).

Russia. We are grateful to Chris Yeates for improving the English language in the manuscript.

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