



## A new species of rust fungi from the middle Eocene Sakhalinian amber

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***Nyssopsora eocaenica* Tykhonenko and Hayova sp. nov., the only fossil species of the genus *Nyssopsora* (Pucciniales), is described from the middle Eocene Sakhalinian amber (Russian Far East). It differs from the other known representatives of the genus by the presence of unbranched hooked appendages on the surface of teliospores. The spores are embedded in the amber sample in close proximity to a syninclusion, *Heterotrissocladius naibuchi* (Diptera, Chironomidae); most of them are clinged to the insect's wing or entangled in the bristles on its legs and body. This fossil is ca. 45 myr old and provides useful information for future phylogeny-based calibration and dating of the rust fungi.**

### Introduction

The order Pucciniales (rust fungi) currently accommodates about 7800 species of obligate parasites of vascular plants (Kirk et al. 2008). According to the most recent phylogenetic research, rust fungi have separated from their closest relatives in Jurassic, at around 175 Ma (Aime and McTaggart 2020). Due to scarce fossil data, the time of origin of the order Pucciniales and divergence dates for its main clades were calibrated mainly by the age of the host plants and fossil evidences of other groups of fungi (McTaggart et al. 2016; Aime et al. 2018). The *Nyssopsora* Arthur, 1906, was established to accommodate the species with triquetrous teliospores bearing appendages on their surface (Arthur 1906). According to modern views, it belongs to the suborder Urediniae but its taxonomic position within this taxon is not confidently resolved and the genus is not yet assigned to any family (Aime and McTaggart 2020). Currently, *Nyssopsora* comprises 10 species of autoecious parasites of plants of the orders Apiales and Sapindales. The present-day geographic range of the genus includes all continents except Africa and Antarctica; the centre of species diversity is located in East Asia, where six species of *Nyssopsora* are reported. Three of four species of *Nyssopsora* parasiting plants of Sapindales are confined to East and South Asia. *Nyssopsora echinata* reveals a prominent pattern of disjunctive distribution between Europe and western North America. In the Eocene, North Atlantic became sufficiently wide to make extremely difficult any direct migration of plants and associated obligate

parasites between the continents (Milne and Abbott 2002); it therefore implies that *N. echinata* may have originated in the Eocene or earlier. More information on the extant species of the *Nyssopsora* is available in publications by Lohsomboon et al. (1990) and Carvalho Junior et al. (2014).

Over the last decade, Sakhalinian amber has yielded numerous fossil arthropods of various taxa (e.g., Simutnik 2014, 2015; Fedotova and Perkovsky 2016; Radchenko and Perkovsky 2016; Marusik et al. 2018; Dietrich and Perkovsky 2019; Kazantsev and Perkovsky 2019; Davidian et al. 2021; Perkovsky et al. 2021; Simutnik et al. 2021 and references therein). In our previous publication (Tykhonenko et al. 2021), we reported the finding of few teliospores of rust fungus similar to those of the modern species *Nyssopsora trevesiae* (Gäumann, 1921) Tranzschel, 1925 in a sample of Sakhalinian amber. Here we describe a new species of the fossil rust fungus from another sample of Sakhalinian amber. For information on the age, outcrop, and fossils from the Naibuchi Formation, where the specimen of Sakhalinian amber was found in situ, see Kodrul (1999).

*Institutional abbreviations.*—PIN, Borissiak Paleontological Institute, Moscow, Russia.

*Nomenclatural acts.*—This published work and the nomenclatural act it contains, have been registered in Index Fungorum: IF558769.

### Material and methods

The type of *Nyssopsora eocaenica* Tykhonenko and Hayova sp. nov. is embedded in a small (7×4×3 mm) clear piece of amber. In the previously published article (Baranov et al. 2015), the inventory number of synincluded holotype of *Heterotrissocladius naibuchi* Baranov, Andersen, and Perkovsky, 2015, was erroneously indicated as PIN 3387/150; the correct number is PIN 3387/147a. Photos were taken with an Olympus CX-41 transmitted light microscope (10× and 20× lenses), with additional illumination from above with a Motic fiber optic illuminator, and an Infinity 2-2 digital camera.

The specimen PIN 3387/147 is deposited in the A.A. Borissiak Paleontological Institute, Russian Academy of Sciences, Moscow.

## Systematic palaeontology

Kingdom Fungi Moore, 1980

Division Basidiomycota Moore, 1980

Class Pucciniomycetes Bauer, Begerow, Sampaio, Weiss, and Oberwinkler, 2006

Order Pucciniales Caruel, 1881

Suborder Uredinineae Engler, 1892 emend. Aime and McTaggart, 2020

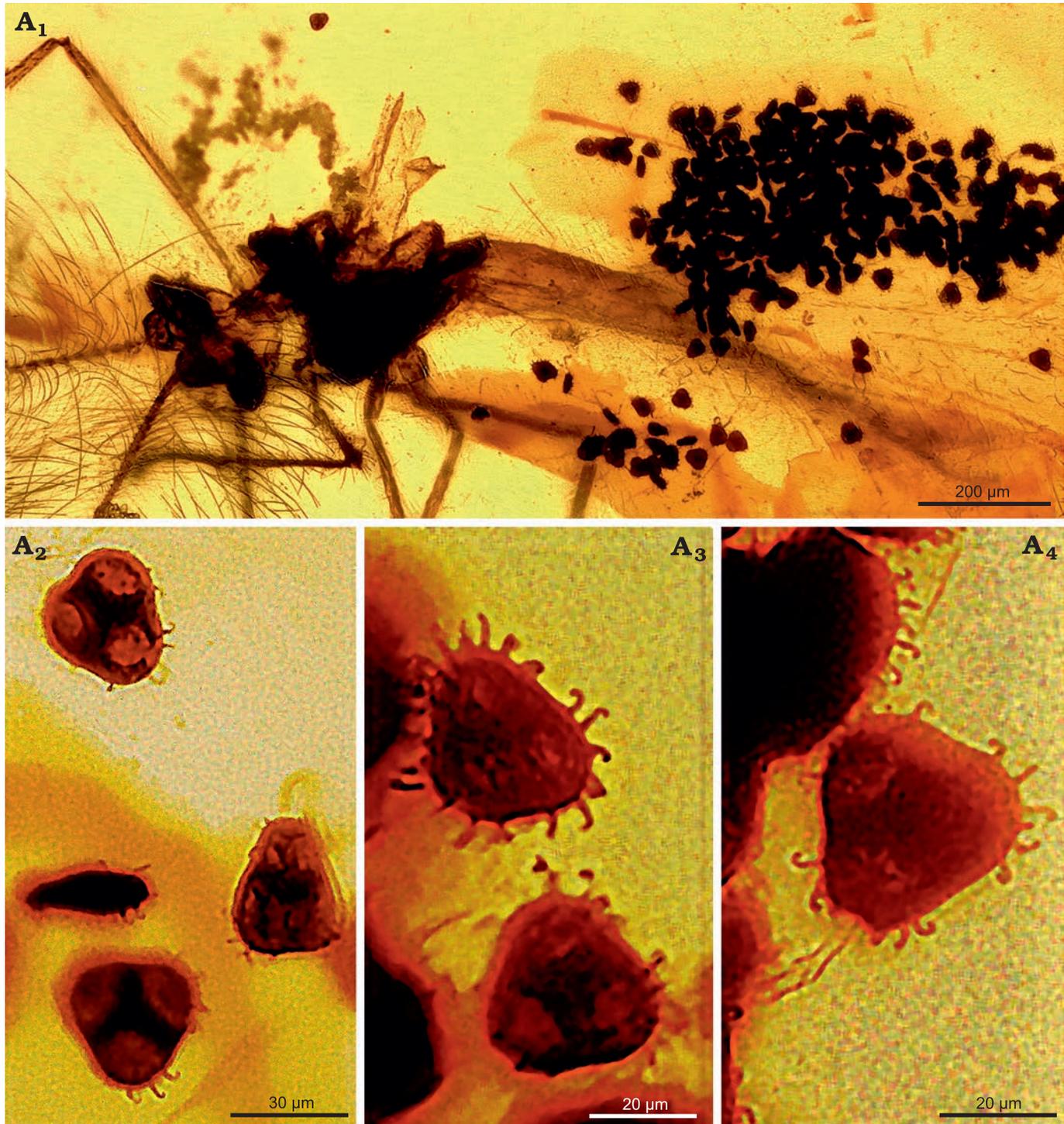
Genus *Nyssopsora* Arthur, 1906*Type species: Nyssopsora echinata* (Léveillé, 1848) Arthur, 1906; Recent, Europe and western North America.

Fig. 1. Rust fungus *Nyssopsora eocaenica* Tykhonenko and Hayova sp. nov., holotype PIN 3387/147 Starodubskoye, Sakhalin, Russia; middle Eocene Sakhalinian amber, Naibuchi Formation. General view of teliospores and part of the holotype of chironomid dipteran *Heterotrissocladius naibuchi* Baranov, Andersen, and Perkovsky, 2015 (A<sub>1</sub>), teliospores with appendages (A<sub>2</sub>, A<sub>3</sub>, A<sub>4</sub>).

*Nyssopsora eocaenica* Tykhonenko and Hayova sp. nov.

Fig. 1.

*Index Fungorum*: IF558769

*Etymology*: Feminine adjective from Eocene, referring to its occurrence in that epoch.

*Holotype*: PIN 3387/147 (more than 200 detached teliospores).

*Type locality*: Starodubskoye village, Dolinsky District, Sakhalin Oblast, Russia.

*Type horizon*: Sakhalinian amber, Naibuchi Formation, middle Eocene, 48.6–40.4 Ma.

*Diagnosis*.—It is easily distinguishable from other hitherto described species of the *Nyssopsora* by the presence of unbranched hooked appendages on the surface of teliospores.

*Description*.—Teliospores consist of 3 cells; the basal one is slightly larger than each of the other two which are both the apical cells. The contours resemble almost equilateral triangles with rounded corners, moderately constricted at the septa (Fig. 1). Spores are coloured, but translucent, so that the septa are clearly visible. Spore sizes vary within 33–38 × 34–37 μm. Pedicels of teliospores are not observed. The outer walls of all cells are covered with numerous simple appendages up to 6–7 μm long, hooked at the apex (Fig. 1). Host plant is unknown.

*Remarks*.—In the article by Baranov et al. (2015), particles adjacent to the holotype of the non-biting midge, *Heterotrissocladius naibuchi* Baranov, Andersen, and Perkovsky, 2015, were considered as angiosperm pollen, but our further careful examination has revealed that undoubtedly they are teliospores of the Pucciniales fungus.

Studying of detached fungal spores is always hard because the absence of sporogenous structures and associated substrate makes correct identification extremely difficult. It is a serendipity that morphological features of the spores in the specimen PIN 3387/147 are sufficient for reliable identification of the fungus. Indeed, due to triquetrous teliospores with appendages it can be assigned to *Nyssopsora* with enough confidence, whereas unbranched and hooked at the tips appendages clearly distinguish the fossil from all previously described species of the extant genus. Since there are no plant inclusions in the amber sample, no reliable assumption about a host plant of *Nyssopsora eocaenica* Tykhonenko and Hayova sp. nov. can be made.

The left wing membrane of *Heterotrissocladius naibuchi* (the right wing is lost) is densely covered with setae (Baranov et al. 2015) and about 80% of all teliospores visible in the specimen PIN 3387/147 are clinged to the wing (Fig. 1A<sub>1</sub>). The position of the spores corroborates the assumption by Savile (1989) that appendages on the surface of the teliospores of rust fungi contribute to the inadvertent transfer of spores by entanglement in the bristles on the legs and body of insects.

Taxonomic identity of the *Nyssopsora* teliospores reported in our recent publication (Tykhonenko et al. 2021) remains unclear. PIN 3387/147 and PIN 3387/973 are both collected in the same locality and originated from the same stratigraphic horizon; however, we cannot claim conspecificity of teliospores

in PIN 3387/147 and PIN 3387/973 due to a small number of spores in the latter and due to a rather uncertain morphology of their appendages.

*Stratigraphic and geographic range*.—Type locality and horizon only.

## Concluding remarks

The age of the amber sample containing *Nyssopsora eocaenica* Tykhonenko and Hayova sp. nov. is reliably determined as ca. 43–47 Ma (Radchenko and Perkovsky 2016). It indicates that *Nyssopsora* must have originated before that time, which is in concordance with current estimation of age (53–83 Ma) for the most recent common ancestor of *Nyssopsora*, Sphaerophragmiaceae and Pucciniaceae (Aime and McTaggart 2020). Thus information on this newly described middle Eocene-aged species of rust fungi can provide some evidence and contribute to estimation of divergence times for the clades of the suborder Urediniales and the order Pucciniales as a whole in future phylogenetic analyses.

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