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Aspicilia stalagmitica (Megasporaceae) – a new lichen species with isidia-like thalline outgrowths

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Summary. *Aspicilia stalagmitica* Paukov et Davydov from the Altai Mts, a species with isidia-like outgrowths on areoles, is described as new to science. From other species of the genus *Aspicilia stalagmitica* differs by the following set of characters: short narrow marginal lobes, conidiomata in the isidia-like outgrowths, appressed to almost substipitate apothecia, long picnoconidia, and stictic acid as a main secondary metabolite. A phylogenetic analysis of *Aspicilia stalagmitica* (ITS) showing its relationships within *Aspicilia* is presented.

Aspicilia stalagmitica (Megasporaceae) – новый вид лишайника с изидиевидными выростами таллома

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Ключевые слова: Алтай, аспицилия, Китай, молекулярная филогения, новый таксон, Синьцзян, ITS.

Аннотация. *Aspicilia stalagmitica* Paukov et Davydov – лишайник из северного Китая с изидиевидными выростами на ареолах, описан как новый для науки вид. От других видов рода он отличается следующей совокупностью признаков: короткие и узкие краевые лопасти, изидиевидные выросты, содержащие конидии, сидячие апотеции, длинные пикноконидии и стиктовая кислота в качестве основного вторичного метаболита. Представлены результаты филогенетического анализа с использованием ITS последовательностей, которые показывают положение вида в пределах рода *Aspicilia*.

Introduction

Family Megasporaceae (or *Aspicilia* s. l.) is remarkable in its morphological diversity and comprises taxa of different life forms from those having immersed thalli to dwarf-fruticose and vagrant species (Sohrabi et al., 2013). Saxicolous and some terricolous representatives of the fruticose life form start their ontogenesis from formation of areolate thalli followed by the development of straight or contorted, simple or branched outgrowths up to several millimeters high (Oxner, 1971). A small number of *Aspicilia* species develop only tiny isidia-like structures which subsequently never form

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fruticose thalli (Poelt, 1961). During field work in the northern China in 2005 Evgeny Davydov collected several specimens with such morphology, which were recognized as representatives of a previously undescribed species. Here we describe this species as new to science.

Materials and Methods

Specimens and phenotype studies

The core material for this study was collected by Evgeny Davydov during the expedition to Xinjiang (China) in 2005 and deposited in herbaria LE, ALTB, UFU, and PE. Morphological observations were made using a dissecting microscope. Cross-sections of apothecia and thalli were cut by hand with a razor blade and observed after mounting in water, K, N and iodine solutions. Measurements of spores and conidia are presented as follows: (smallest value recorded) (X-SE) – $\underline{X}$ – (X+SE) (largest value recorded), where $\underline{X}$ is the (arithmetic) sample mean, and SE the sample error of mean. The measurements were made with the precision of 0.5 µm.

Secondary products were analyzed by applying standard thin-layer chromatography techniques (TLC, Culberson, Kristinsson, 1970). Solvent A (toluene : 1,4-dioxane : acetic acid, 180 : 45 : 5) and C (toluene : acetic acid, 170 : 30) systems were used for the TLC analysis.

Sequences and phylogenetic reconstructions

To test phylogenetic relations to other species, nuclear internal transcribed spacers and 5.8S rDNA (ITS) sequences of the putative new species and other sequences retrieved from the NCBI database (GenBank) were used for a molecular phylogenetic analysis. Our sampling comprised 14 species of *Aspicilia* including a putative new species, species of *Oxneriaria, Lobothallia* and *Circinaria*, as well as *Ochrolechia parella* (L.) A. Massal as an outgroup. This selection is based on the studies of Nordin et al. (2007, 2008, 2010), Kondratyuk et al. (2016), Haji Moniri (2017) and a five-gene analysis by Miądlikowska et al. (2014). The information on the samples with the GenBank accession numbers are given in Table.

Table

Species of lichens used in the phylogenetic analysis in this study together with specimen information and GenBank Accession numbers. New specimen and its sequence are in bold.

Species	Origin	Collection number or reference	ITS GenBank Accession number	Reference
Aspicilia abbasiana	China	Ismayil et Abbas 20111154	KM609324	Ismayil et al., 2015; Kondratyuk et al., 2016
A. berntii	Norway	Nordin 6392	EU502747	Nordin et al., 2008
A. blastidiata	Russia	Paukov AGP20111009-01	KX129963	Paukov et al., 2015, 2017
A. blastidiata	Russia	Paukov AGP20120801-01	KX159286	Paukov et al., 2015, 2017
A. cinerea	France	Roux 23869	JF703118	Roux et al., 2011
A. cinerea	France	Roux 25015	JF710311	Roux et al., 2011
A. cuprea	USA	Owe-Larsson 9112	EU057902	Nordin et al., 2007
A. dudinensis	Sweden	Nordin 6036	EU057906	Nordin et al., 2007
A. epiglypta	Sweden	Nordin 6303	EU057907	Nordin et al., 2007
A. epiglypta	Sweden	Nordin 6305	HQ259261	Nordin et al., 2011
A. fluviatilis	Sweden	Nordin 6188	HQ259264	Nordin et al., 2011
A. goettweigensis	Austria	Vondrák 14026	KX159289	Paukov et al., 2017
A. goettweigensis	Russia	Paukov AGP20120513-03	KX159292	Paukov et al., 2017
A. granulosa	Sweden	Nordin 6174	HQ259265	Nordin et al., 2011
A. stalagmitica	**China**	**Davydov 17620**	**MT014019**	**This paper**
A. subdepressa	France	Roux 24653	JF703123	Roux et al., 2011
A. subepiglypta	Korea	100857 KoLRI	KY249607	Kondratyuk et al., 2016
A. subepiglypta	Korea	110495 KoLRI	KY249608	Kondratyuk et al., 2016
A. subradians	Sweden	Nordin 5984	HQ259267	Nordin et al., 2011
A. subradians	Finland	Nordin 6370	HQ259268	Nordin et al., 2011
A. verrucigera	Sweden	Tibell 22669	EU057939	Nordin et al., 2007
Circinaria esculenta	Kazakhstan	Ivanov s. n. (UFU L-1743)	MK347507	Paukov et al., 2019

Table (end)

Species	Origin	Collection number or reference	ITS GenBank Accession number	Reference
C. fruticulosa	Russia	Paukov 3074 (UFU L-3256)	MK347508	Paukov et al., 2019
Lobothallia alphoplaca	China	Tong 20117616 (SDNU)	JX499233	Kou et al., 2013
L. praeradiosa	China	Mamut s. n. (XJU)	KT180160	Ismayil, Abbas not published
L. praeradiosa	Russia	Paukov AGP20120606-12 (UFU L-1264)	MK347501	Paukov et al., 2019
Ochrolechia parella	Antarctica	Park PCH080112-32	KJ607905	Park et al., 2015
Oxneriaria dendroplaca	Finland	Nordin 6366	HQ259260	Nordin et al., 2011
O. mashiginensis	Sweden	Nordin 5790	EU057912	Nordin et al., 2007
O. permutata	Sweden	Nordin 6039	EU057921	Nordin et al., 2007
O. permutata	Sweden	Nordin 5980	EU057930	Nordin et al., 2008
O. rivulicola	Sweden	Nordin 5957	EU057922	Nordin et al., 2007
O. supertegens	Norway	Owe-Larsson 9002	EU057936	Nordin et al., 2007
O. supertegens	Sweden	Nordin 6023	EU057938	Nordin et al., 2007
O. verruculosa	Norway	Owe-Larsson 9007	EU057940	Nordin et al., 2007
O. verruculosa	Sweden	Nordin 5942	EU057942	Nordin et al., 2007
O. virginea	Sweden	Nordin 6017a	HQ259270	Nordin et al., 2011
O. virginea	Svalbard	Ebbestad SvL1:1	HQ259271	Nordin et al., 2011

Methods used for DNA extraction, amplification and sequencing follow Davydov et Yakovchenko (2017). An ITS 534 bp matrix were aligned using the MAFFT algorithm (Katoh et al., 2005) as implemented on the GUIDANCE web server (Sela et al., 2015). Optimal substitution models were inferred separately for ITS1, 5.8S, and ITS2 using PartitionFinder, version 1.1.1 (Lanfear et al., 2012): the General time reversible parameter with gamma distribution site specific rates (GTR+G) for the ITS1+ITS2 partition, and the Kimura 2-parameter with proportion of invariable sites (K80+I) for the 5.8S partition. Bayesian inference with the Markov chain Monte Carlo (BMCMC) method (Larget, Shimon, 1999) was performed using MrBayes 3.2.3 (Ronquist et al., 2012). Three parallel Bayesian analyses were run using six chains and every 200th generation was sampled. Convergence of the chains was inferred by calculating the average standard deviation of split frequencies every 100,000 generations using a burn-in fraction of 0.5, and the runs terminated when the standard deviation of split frequencies dropped below 0.001. This was the case after 7.1M generations. The first 50 % of the trees were discarded as burn-in and a 50 % majority rule consensus tree calculated from the remaining trees of three runs with the sumt command implemented in MrBayes 3.2.3. The most likely tree and 1000 rapid bootstrap replicates were calculated using RAxML 8.0.26 (Stamatakis, 2014) by raxmlGUI software version 1.3.1 (Silvestro, Michalak, 2012) applying the GTRGAMMA model of substitution to the subsets. The tree topologies were taken from RAxML. Bootstrap support values and BMCMC posterior probability were noted onto the best scoring tree (Fig. 1).

Results

An ITS sequence was successfully obtained from one specimen of the putative new species, described below as *Aspicilia stalagmitica*. The Bayesian 50 % majority-rule consensus tree had the same topology as the maximum likelihood tree generated by RAxML. The phylograms are combined in Fig. 1. According to the ITS sequence the new taxon belongs to *Aspicilia* and is the closest relative to the North-American *Aspicilia cuprea* Owe-Larss. et A. Nordin. These two taxa form a clade well-supported by MrBayes (0.97PP), but only weakly by RAxML (62 % BS) and rather long branches lengths. The sister clade contains the type species of the genus, *Aspicilia cinerea* (L.) Körb.

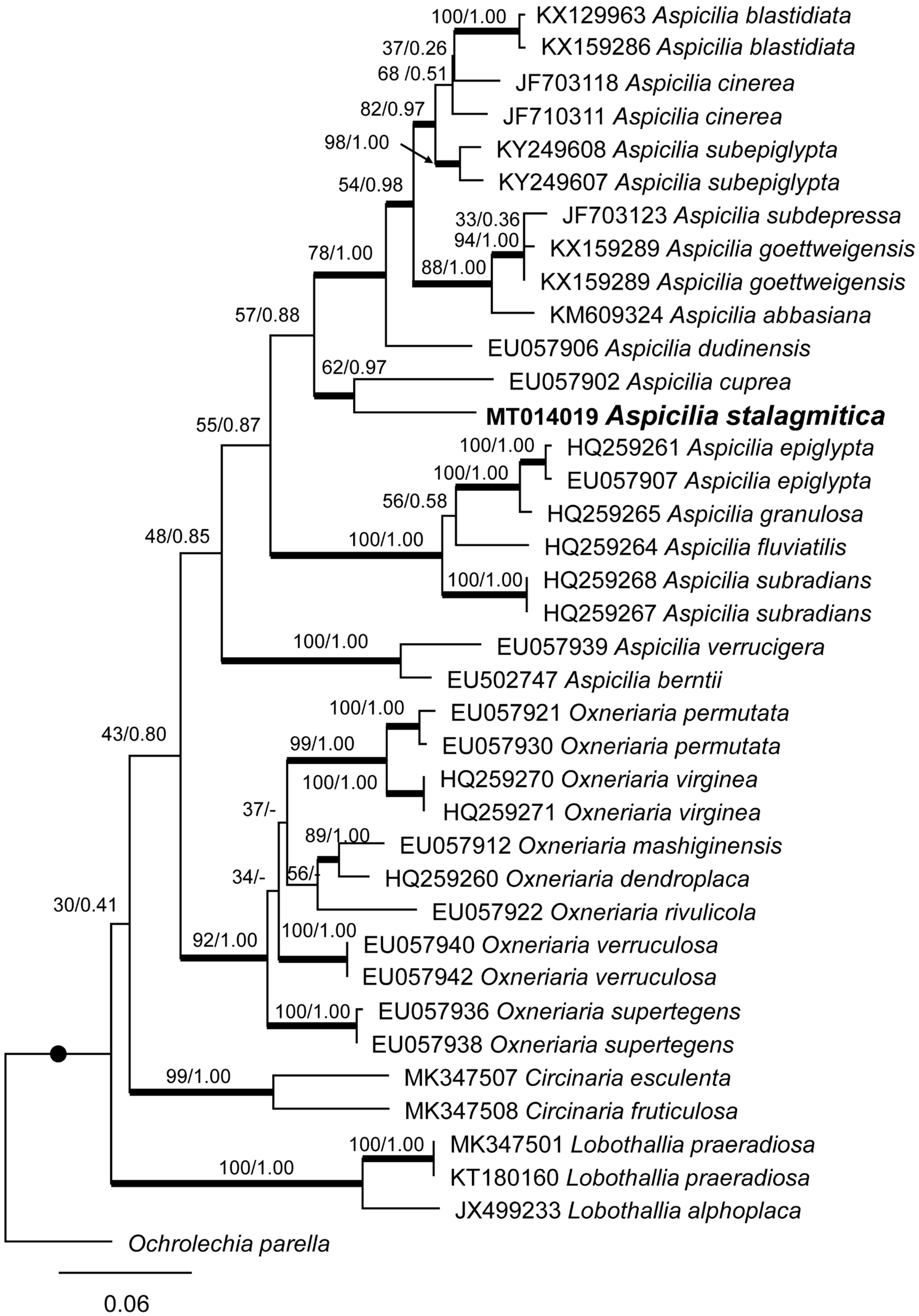

Fig. 1. Maximum likelihood (ML) phylogeny of selected *Aspicilia* ITS sequences. The reliability of each branch was tested by ML and Bayesian methods. Numbers at tree nodes indicate ML bootstrap percentages (left) and Bayesian inference with the Markov chain Monte Carlo (BMCMC) posterior probabilities (right). Thicker branches indicate when the bootstrap value of ML is ≥ 70 % or the BMCMC posterior probability is ≥ 0.95. Accession numbers are given to serve as operational taxonomic unit (OTU) names (see Table). Originally produced sequence is marked in bold. *Ochrolechia parella* was used as an outgroup. Branch lengths represent the estimated number of substitutions per site assuming the respective models of substitution. Exception is the branch with a black dot, which was shortened to reduce the overall figure size.

The species

Aspicilia stalagmitica Paukov et Davydov, **sp. nov.**

MycoBank No.: MB 834291

Aspicilia with a thin, indistinctly lobate, areolate, grey thallus with isidia-like outgrowths usually containing conidiomata. Lobes short and narrow or absent, areoles angular, apothecia sessile, conidia long, 19–33 µm. Main secondary metabolite stictic acid.

Type: "China, Xinjiang, Mongolsky Altai range, SW vicinity of Altai City, granite rocks near the road, 47°47'49"N, 88°04'49"E, elev. 900 m, on rocks. 4 VIII 2005. E. A. Davydov. № 17620" (holo – LE-L15292, iso – ALTB, UFU-L3488) (Fig. 2).

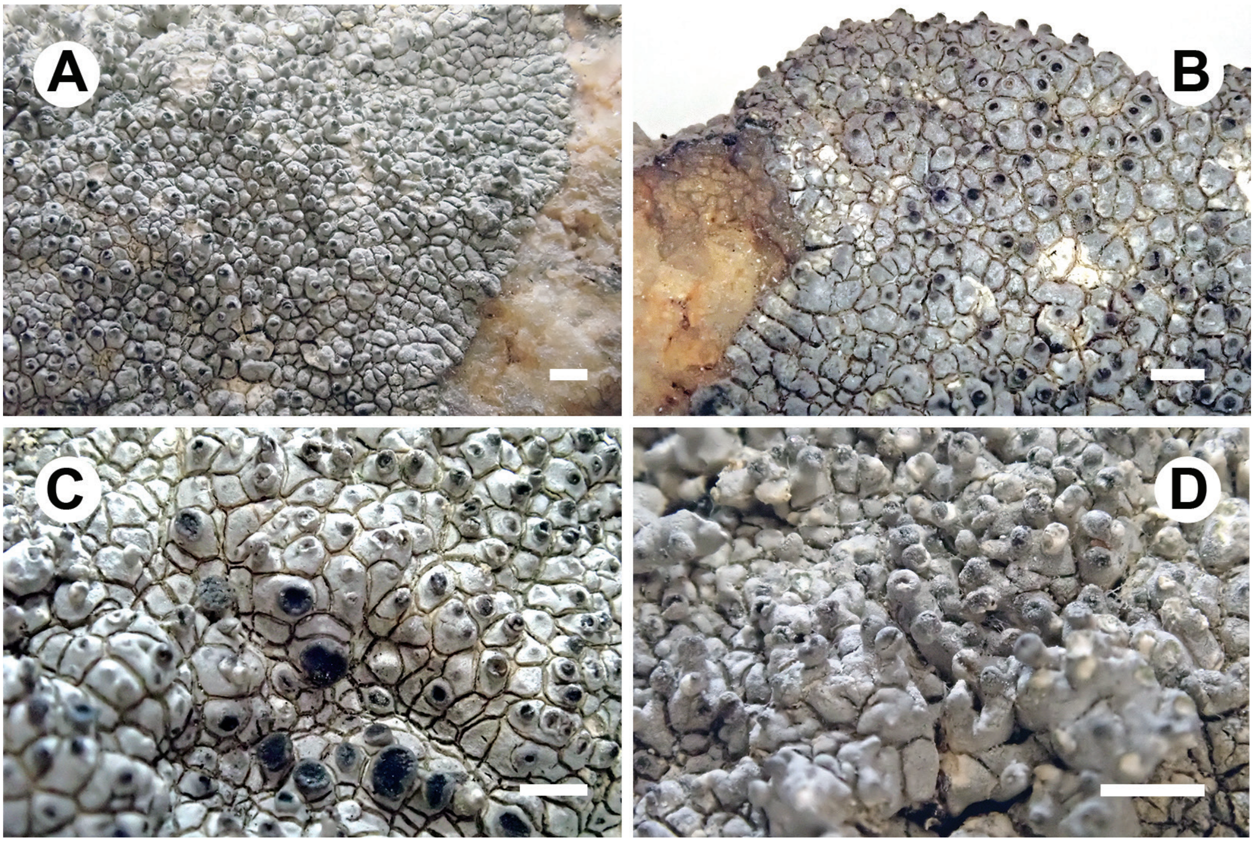

Fig. 2. *Aspicilia stalagmitica* (holotype): A – thallus; B – outer part of the thallus with young projections, C – thallus with apothecia; D – projections in the central part of the thallus. Scale = 1 mm.

Life habit lichenized. Thallus grey, up to 1.5 mm thick, indistinctly lobate at the periphery and areolate in the central part. Lobes relatively short and narrow, 0.5–1.5 × 0.4–0.5 mm (length × width), moderately convex, inconspicuous in some thalli. Areoles 0.5–1.7 mm, irregular in form, angular, moderately convex, with isidia-like outgrowths. The outgrowths are sphaeric, one, two, rarely more per areola, constricted at the bases, 0.25–0.5 mm, brittle, in the central parts of thalli occasionally cylindric, up to 0.75 mm high, blackish at the tops, commonly containing pycnidia with blackish spot-like or elongated ostioles. Upper cortex paraplectenchymatous, 30–50 µm high, cells 7–10 µm. Medulla I−, K+ yellow, with rare needle-like crystals. Photobiont layer 50–70 µm thick, interrupted by narrow hyphal bands 5–10 µm. Photobiont chlorococcoid, algae 7–20 µm diam. Prothallus absent. Vegetative propagules absent. Apothecia lecanorine, 1 per areole, developing from the outgrowths, appressed, later sessile, constricted at the base, rounded or elliptic in outline, 0.3–0.7 mm diam.; disc initially dot-like, later wide, flat, not pruinose or weakly white pruinose, blackish, surrounded by a projecting thalline margin. Margin 0.10–0.15 mm, lead-grey, darker than the thallus. Exciple of radiating hyphae, poorly recognizable under the hypothecium, widening to 25–30 µm in the uppermost part. Hymenium hyaline, 100–112 µm high, fleetingly bluish in I; paraphyses predominantly submoniliform, rarely moniliform with 2–3 apical cells thickened; epihymenium brownish, N+ greenish, 57–62 µm high. Hypothecium hyaline, I+ weakly bluish, 100–120 µm in the central part.

Asci clavate, *Aspicilia*-type; ascospores broadly ellipsoid, hyaline, aseptate (19.0-)21.0–21.5–22.0 (–23.0) × (12.0–)13.5–14.0–14.5(–16.0) µm (n = 10). Pycnidia common, in isidia-like projections, with punctiform or elongated ostiole; conidia bacilliform, hyaline, curved or straight, aseptate (19.0–)24.5–25.0–25.5(33.0) µm long (n = 56).

Chemistry. Thallus K+ yellow, C−; medulla K+ yellow, C−; stictic acid complex by TLC, norstictic acid as a minor substance in all specimens.

Etymology. The name refers to the vertical outgrowths on areoles which resemble stalagmites.

Ecology. *Aspicilia stalagmitica* was found in arid conditions on exposed siliceous rocks (granite and schistose) in steppe communities at elevations 880–1600 m a. s. l. The following species co-occurred with *Aspicilia stalagmitica*: *Acarospora bohlinii* H. Magn., *A. irregularis* H. Magn., *Aspicilia cinerea* (L.) Körb., *Candelariella vitellina* (Ehrh.) Müll. Arg., *Circinaria maculata* (H. Magn.) Q. Ren, *C. hoffmanniana* (S. Ekman et Fröberg ex R. Sant.) A. Nordin, *Immersaria cupreoatra* (Nyl.) Calat. et Rambold, *Lecanora argopholis* (Ach.) Ach., *Protoparmeliopsis garovaglii* (Körb.) Arup et al., *Rusavskia dasanensis* S. Y. Kondr. et al., and *Xanthoparmelia delisei* (Duby) O. Blanco et al.

Distribution. The species is known from three localities in the Xinjiang autonomous region of China.

Paratypes: China, Xinjiang, Mongolsky Altai range, “left bank of the Kran River, Altai City, S slope of Mt., on rocks, elev. 880 m. 47°49'52"N, 88°08'08"E. 31 VII 2005. E. A. Davydov. № 18943” (ALTB, UFU); “valley of Irtysh River at 10 km E settlement Kektogoy, steppe slope, on rocks, elev. 1300–1600 m. 47°13'40"N, 89°55'18"E. 7 VIII 2005. E. A. Davydov. № 17618, 17619” (ALTB, UFU).

Discussion

Aspicilia stalagmitica is a peculiar species which can be easily distinguished from other *Aspicilia* s. l. by its isidia-like outgrowths, which contain conidiomata. Along with this character the species has long pycnoconidia, up to 33 µm, and contains the stictic acid complex. All the found specimens contain vertical outgrowths but the combination of long conidia and stictic acid may be a separate character which segregates this species from all known taxa within the genus.

According to the ITS phylogeny the closest species to *Aspicilia stalagmitica* is *A. cuprea*, which is known from the USA, California. It differs from the former by its similarity to *Aspiciliella cupreoglauca* (B. de Lesd.) Zakeri et al. (Zakeri et al., 2019), i. e. its brown thallus, immersed apothecia, and large spores (20–31 × 11–17 µm) (Owe-Larsson et al., 2007). The conidium length is a character, which segregates both *Aspicilia cuprea* and *A. stalagmitica* from the *Aspicilia cinerea*-group. Only *Aspicilia dudinensis* (H. Magn.) Oxner from this group has a conidium length that considerably overlaps with *Aspicilia stalagmitica* and *A. cuprea* (Nordin et al., 2008; Paukov et al., 2017), but never reaches 30 µm. Other similar species with conidia exceeding this range belong to *Oxneriaria* (*Oxneriaria rivulicola* (H. Magn.) S. Y. Kondr. et L. Lőkös, and *O. supertegens* (Arnold) S.Y. Kondr. et L. Lőkös), but lack secondary metabolites.

Compared to species with isidia-like projections, *Aspicilia stalagmitica* is most similar to *Oxneriaria mashiginensis* (Zahlbr.) S.Y. Kondr. et L. Lőkös, which, however, differs by its darker thalli, smaller spores (14–18 × 9–11 µm) and shorter conidia (12.5–19.0 µm), and by containing substictic acid. Further, the outgrowths usually disintegrate into soredia and do not contain conidiomata (Nordin et al., 2008).

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