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ORIGINAL ARTICLE

Atrostelia—a new genus in *Megasporaceae* (*Pertusariales*, lichenized *Ascomycota*)

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Abstract

Phylogenetic analysis based on nu ITS, nu LSU, and mt SSU ribosomal DNA fragments revealed a new genus in *Megasporaceae* described here as *Atrostelia* Paukov, Davydov & Yakovchenko. Phylogenetically, it is close to *Aspiciliella* but differs in radiate-placodioid thalli, large, 23–36 µm spores, and substictic acid as a secondary metabolite. The genus is represented by a single species, *Atrostelia magnifica* Paukov, Davydov & Yakovchenko, which is endemic to the Mongun Taiga massif, Republic of Tyva, Russia. The phylogenetic reconstruction supported 9 genera in *Megasporaceae*: *Aspicilia*, *Aspiciliella*, *Atrostelia*, *Circinaria*, *Lobothallia*, *Megaspora*, *Oxneriaria*, *Sagedia*, and *Teuvoa,* while *Agrestia*, *Chlorangium*, and *Sphaerothallia* were synonymous with *Circinaria*.

Keywords Altai Mts. · nu ITS · nu LSU · mt SSU · Taxonomy · 1 new species

Introduction

The family *Megasporaceae* was erected to accommodate the genus *Megaspora* (Clauzade & Cl. Roux) Hafellner & V. Wirth in *Pertusariales* instead of *Hymeneliaceae* (*Lecanorales*) (Lumbsch et al. 1994). The family temporarily remained monotypic, but with the use of molecular markers, it was shown that the genus *Aspicilia* s.l. is much closer to *Megaspora* than to other representatives of *Pertusariales* (Ivanova and Hafellner 2002) which enlarged the concept of *Megasporaceae* by including *Aspicilia* Massal. and *Lobothallia* (Clauzade & Cl. Roux) Hafellner into the family (Schmitt et al. 2006). Nordin et al. (2010) supported the monophyly of *Megasporaceae*, leaving a branch containing *Aspilidea* Hafellner, '*Variolaria*' (*Lepra* Scop.) and *Varicellaria* Nyl. as a sister to *Megasporaceae*, resurrected *Circinaria* Link, *Sagedia* Ach., and recircumscribed *Lobothallia,* including the subgenus *Pachyothallia* Clauzade & Roux. In the same article, *Aspicilia* was subdivided into two branches. The first branch containing the type species *Aspicilia cinerea* Körb. had low bootstrap support, while the second, including *Aspicilia mashiginensis* (Zahlbr.) Oxner and *A. supertegens* Arnold, was highly supported and described later as a genus *Oxneriaria* S.Y. Kondr. & Lőkös (Haji Moniri et al. 2017). Some older genera, *Agrestia* J.W. Thomson, *Chlorangium* Link and *Sphaerothallia* Nees ex Eversm. were resurrected

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by the same authors from *Circinaria* to accommodate three supported clades of the phylogenetic tree (Kondratyuk et al. 2015). The genus *Teuvoa* Sohrabi & S. Leavitt was proposed for corticolous and lignicolous species of *Aspicilia* s.l. These species were similar to *Lobothallia* in the size of spores and conidia but lacked thalline lobes and a subhypothecial algal layer (Sohrabi et al. 2013a). The genus *Aspiciliella* M. Choisy in Werner was proposed in 1932 (Werner 1932) but was not widely accepted and resurrected only recently (Zakeri et al. 2017). It differs from *Aspicilia* by its larger spores, short conidia, the presence of norstictic acid in all species and by a different position in the phylogenetic tree of the family. Generally, representatives of 35 genera were regarded as *Aspicilia* s.l. Of them, 14 genera are illegitimate, 10 currently belong to other families, and 11 genera belong to *Megasporaceae* (Fig. 1).

During the study of the lichen diversity of the Mongun-Taiga massif E.D. and L.Y. collected specimens that had a set of morphological and anatomical features not characteristic of any known genus in *Megasporaceae*. The thick thallus and the large spores were similar to *Circinaria,* but unlike the most species in *Circinaria* the specimens had 8 spores in ascus. Furthermore, the thalli had a placodioid life form similar to *Lobothallia*, younger apothecia were immersed and had black discs, as in *Aspiciliella.* The secondary metabolite was substictic acid, which is common in *Oxneriaria*. Multilocus phylogeny revealed the position of the specimens as a robust branch relative to *Aspiciliella*. Here, we describe them as a new genus, *Atrostelia,* in *Megasporaceae*.

Materials and methods

Sampling and phenotype studies

Morphological and anatomical characteristics were analyzed by applying standard light microscopic methods. Cross-sections of apothecia and thalli were sectioned by hand with a razor blade and observed after mounting in water. Measurements are given as follows: (smallest value recorded) $X - \mathrm{SD} - [X] - X + \mathrm{SD}$ (largest value recorded), where X is the (arithmetic) sample mean and SD is the sample standard deviation. The two extreme values and the sample mean are given to the nearest 0.5 µm.

Table 1 Primers used in this study

Primers	5′–3′ sequence	Reference
ITS1-F	CTTGGTCATTTAGAGGAAGTAA	Gardes & Bruns 1993
ITS4	TCCTCCGCTTATTGATATGC	White et al. 1990
LROR	ACCCGCTGAACTTAAGC	Vilgalys lab unpublished
LR3	CCGTGTTTCAAGACGGG	Vilgalys lab unpublished
mtSSU1	AGCAGTGAGGAATATTGGTC	Zoller et al. 1999
mtSSU3R	ATGTGGCACGTCTATAGCCC	Zoller et al. 1999

Secondary metabolites present in the thallus were examined using high-performance thin layer chromatography (HPTLC) (Culberson and Kristinsson 1970). Solvent systems A, B′ and C (Culberson and Johnson 1982) were used for HPTLC analysis.

Sequences and phylogenetic reconstructions

DNA extraction, amplification, and sequencing were performed as described in Davydov and Yakovchenko (2017). The program Geneious 6.0 (Biomatters Ltd., New Zealand) was used for assembling sequence reads and datasets. To test the phylogenetic relationships of the putative new taxon, the internal transcribed spacer region of nuclear ribosomal DNA (nu ITS), the large subunit of nuclear ribosomal DNA (nu LSU), and the small subunit of mitochondrial ribosomal DNA (mt SSU) were sequenced from two specimens. All primers used in this study are shown in Table 1. Sequences

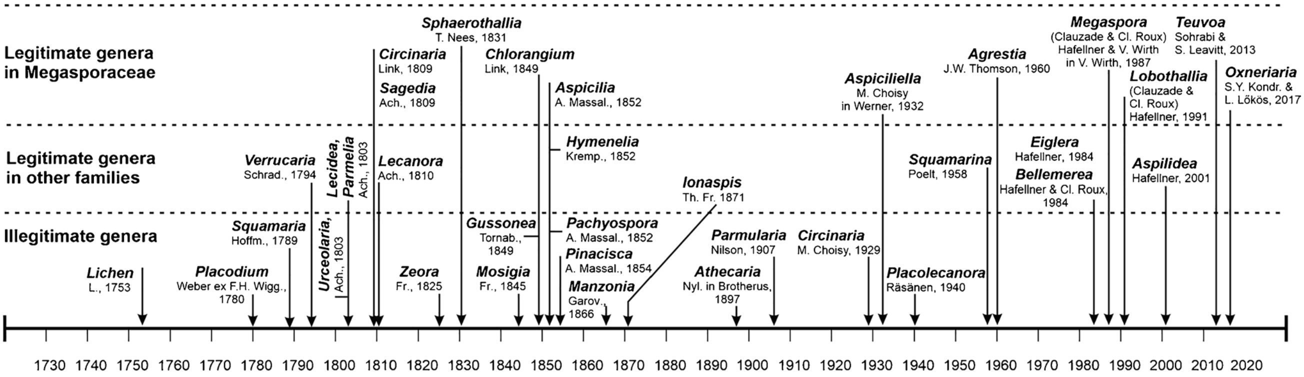

Fig. 1 Genera, which representatives were regarded as *Aspicilia* s.l., their authors, and years of description

were aligned with those of 42 species, representing eight genera of *Megasporaceae*, preferable from the most comprehensive phylogenetic study of the family by Nordin et al. (2010), Sohrabi et al. (2013a), and other recent studies (Kondratyuk et al. 2015; Haji Moniri et al. 2017; Sohrabi et al. 2013b). In addition, we produced 20 sequences of 7 specimens of *Atrostelia magnifica*, *Circinaria affinis*, *C. fruticulosa*, *C. schafeevii*, *C. tortuosa*, and *Lobothallia praeradiosa*. Accession numbers are provided in Table 2. The abovementioned markers were used because they were also used in phylogenetic analyses of *Megasporaceae* by Schmitt et al. (2001), Nordin et al. (2007, 2010), Owe-Larsson et al. (2011), Sohrabi et al. (2013a, b), as well as many sequences available from GenBank, whereas other loci are available only for very few species.

Three single-gene datasets containing the sequences were compiled and aligned using the MAFFT algorithm (Katoh et al. 2019). Introns in the mtSSU and nuLSU sequences were removed from the alignments. Before combining sequences into a joint nc ITS + mt SSU + nc LSU data matrix, the unambiguously alignable regions were used to calculate single-marker phylograms (not shown) using RAxML 8.0.26 (Stamatakis 2014), which were tested for conflicts among data sets. Because the cladograms were similar regarding well-supported clades and therefore lacked conflicts, all sequences were combined into one matrix consisting of 1616 sites and used for maximum likelihood and Bayesian analyses. *Ochrolechia balcanica* Verseghy was used as an outgroup, following a previously published analysis of *Pertusariales* (Schmitt et al. 2001).

A heuristic search for the maximum likelihood (ML) bootstrap tree with simultaneous inference of the optimal partitioning scheme and substitution models for each data partition was performed using the online version of IQ-TREE (Nguyen et al. 2015; Trifinopoulos et al. 2016), suggesting five initial partitions (nu ITS1, 5.8S rDNA, ITS2, mt SSU, and nu LSU). Branch lengths were assumed to be equal for all partitions. Branch support was estimated with the ultrafast bootstrap algorithm (Minh et al. 2013) based on 1000 bootstrap replicates and using a maximum of 1000 iterations and a minimum correlation coefficient of 0.99 as a stopping rule (bootstrap support, BS). Another ML tree and 1000 bootstrap replicates were computed using RAxML (Stamatakis 2014) with raxmlGUI software version 1.5 (Silvestro and Michalak 2012), applying the GTRGAMMA substitution model to the subsets, as RAxML does not support HKY and K80 models.To provide additional support for our phylogenetic reconstruction, we ran Bayesian analysis. We used the Markov chain Monte Carlo method (Larget and Simon 1999) implemented in MrBayes 3.2.3 (Ronquist et al. 2012) to infer phylogenetic trees by applying the partitioning scheme inferred with IQ-TREE and slightly simplified substitution models inferred by PartitionFinder, ver. 1.1.1 (Lanfear et al. 2012) because most of the models inferred by IQ-TREE are not implemented in MrBayes (Table 3). Three parallel analyses, each with 6 incrementally heated chains using the default heating factor of 0.2, were run for 100 million generations, and every 200th generation was sampled. The burn-in was determined using Tracer (v. 1.7.2) (Rambaut et al. 2018). The first 5% of the trees were discarded as burn-in, and a 50% majority rule consensus tree was calculated from the remaining trees of the three runs with the sumt command implemented in MrBayes 3.2.3. Newly generated DNA sequences were uploaded to GenBank.

Results

The maximum likelihood and Bayesian analyses of the combined dataset had identical topologies (Fig. 2) and were similar to those in Nordin et al. (2010) and Sohrabi et al. (2013a), except for the position of *Sagedia*, which forms a supported branch different from the morphologically similar *Aspicilia* and *Oxneriaria*. This fact contradicts the conclusion that *Sagedia* should be included in *Aspicilia* (Miadlikowska et al. 2014), and we consider them separate genera. The position of *Sagedia* in the tree of *Megasporaceae* remains, however, not fully resolved, as the Bayesian posterior probability (PP) of the branch containing the rest of the genera was lower than a threshold level of 0.95. Otherwise, well-supported pairs of genera were revealed within this branch. Similar to the phylogenies reconstructed in Nordin et al. (2010) and Sohrabi et al. (2013a), *Lobothallia* is sister to *Teuvoa*, *Aspicilia* is sister to *Oxneriaria*, and *Megaspora* is sister to *Circinaria*. Sequences of type species of *Agrestia* (*Agrestia cyphellata* J. W. Thomson = *Circinaria hispida* (Mereschk.) A. Nordin, S. Savić & Tibell), *Chlorangium* (*Chlorangium jussuffii* (Link) Link), and *Sphaerothallia* (*Sphaerothallia esculenta* (Pall.) Eversm. in T. Nees) (Fig. 2) did not form well-defined lineages, and we consider them synonyms of *Circinaria*. The analysis revealed *Atrostelia* a sister genus of *Aspiciliella*. The position of two species pairs in the tree is well resolved. *Megaspora* and *Ciricnaria* together with *Aspiciliella* and *Atrostelia* form a supported branch (IQ BS/RAxML BS/PP = 99/95/1.0) that is characterized by large spores, 25–65 µm in contrast to other genera where spores rarely exceed 25 µm.

Taxonomy

Atrostelia magnifica Paukov, Davydov & Yakovchenko, **gen. et sp. nov.**, Fig. 3

MycoBank **851229** (genus) **851231** (species).

Differs from *Aspiciliella* by the radiate-placodioid thalli, larger spores, substictic acid as a secondary metabolite and the distribution in mountain tundras. From the

Table 2 Species of lichens used in the phylogenetic analyses in this study together with specimen information and GenBank accession numbers. New specimens and associated sequences are in bold

Species	Country	Specimen number	GenBank number			Reference
			nc ITS	nc LSU	mt SSU	
Aspicilia cinerea	Sweden	Hermansson 13275	EU057899	HM060733	HM060695	Nordin et al. 2007
Aspicilia cinerea	Sweden	Nordin 5542	HQ406799	HM060734	HM060696	Owe-Larsson et al. 2011
Aspicilia cuprea	USA	Owe-Larsson 9112	EU057902	HM060750	HM060712	Nordin et al. 2007
Aspicilia cyanescens	USA	Owe-Larsson 9151	EU057904	HM060745	HM060707	Nordin et al. 2007
Aspicilia dudinensis	Sweden	Nordin 6036	EU057906	HM060748	HM060710	Nordin et al. 2007
Aspicilia epiglypta	Sweden	Nordin 6303	EU057907	HM060718	HM060756	Nordin et al. 2007
Aspicilia indissimilis	Sweden	Nordin 5943	EU057909	HM060746	HM060708	Nordin et al. 2007
Aspicilia laevata	Sweden	Tibell 23659	EU057910	HM060692	HM060730	Nordin et al. 2007
Aspiciliella cupreoglauca	Greece	Sipman & Raus 61847	KY618843	KY576954	KY576930	Zakeri et al. 2019
Aspiciliella cupreoglauca	Greece	Sipman & Raus 62345	KY618844	KY576955	KY576931	Zakeri et al. 2017
Aspiciliella intermutans	Bulgaria	Vondrák 2151	MH248865	MH248872	MH255568	Zakeri et al. 2019
Aspiciliella intermutans	Greece	Sipman & Raus 61847	MH210648	MH257198	MH257234	Zakeri et al. 2019
Aspiciliella portosantata	Portugal	Sipman 62854	KY618851	KY576961	KY576938	Zakeri et al. 2017
Aspiciliella portosantata	Portugal	Sipman 63025	KY618853	KY576963	KY576940	Zakeri et al. 2017
Atrostelia magnifica **(isotype)**	**Russia**	**Davydov 21959 & Yakovchenko**	**PP832009**	**PP832002**	**PP842134**	**This paper**
Atrostelia magnifica **(paratype)**	**Russia**	**Davydov 21961 & Yakovchenko**	**PP832010**	**PP832003**	**PP842135**	**This paper**
Circinaria affinis	**Russia**	**Paukov 1978**	**PP832011**	**PP832004**	**PP842136**	**This paper**
Circinaria caesiocinerea	Sweden	Tibell 22612	EU057897	HM060731	HM060693	Nordin et al. 2007
Circinaria calcarea	Sweden	Nordin 5888	EU057898	HM060743	HM060705	Nordin et al. 2007
Circinaria 'elmorei'	Russia	Owe-Larsson 9814	HQ406802	HM060727	HM060689	Owe-Larsson et al. 2011
Circinaria emiliae	Kazakhstan	Kulakov 3702	JQ797512	HM060728	HM060690	Sohrabi et al. 2013b
Circinaria esculenta	Russia	Owe-Larsson 9796	JQ797511	JQ797493	JQ797485	Sohrabi et al. 2013b
Circinaria fruticulosa	**Russia**	**Paukov 3354**	**PP832012**	**PP832005**	**PP842138**	**This paper**
Circinaria gibbosa	Sweden	Nordin 5878	EU057908	HM060740	HM060702	Nordin et al. 2007
Circinaria hispida	Turkey	Candan 11	HQ406806	HM060760	HM060722	Owe-Larsson et al. 2011
Circinaria jussuffii	Algeria	Esnault 2033	JQ797518	JQ797495	JQ797489	Sohrabi et al. 2013b
Circinaria lacunosa	China	Abbas 940003	JQ797517	JQ797494	JQ797490	Sohrabi et al. 2013b
Circinaria leprosescens	Sweden	Nordin 5906	EU057911	HM060749	HM060711	Nordin et al. 2007
Circinaria rostamii	Iran	Sohrabi 10212	JQ797538	JQ797507	JQ797491	Sohrabi et al. 2013b
Circinaria schafeevii	**Russia**	**Paukov 1975**	**PP832013**	**PP832006**	**PP842139**	**This paper**
Circinaria tortuosa	**Russia**	**Paukov 1968**	**PP832014**	**PP832007**	**PP842137**	**This paper**
Lobothallia alphoplaca	USA	Leavitt 743	JX306738	KC667059	-	Sohrabi et al. 2013a
Lobothallia alphoplaca	USA	Leavitt 849	JX306739	KC667060	-	Sohrabi et al. 2013a
Lobothallia brachyloba	Russia	Frolov 357	MK347506	-	MK348228	Paukov et al. 2019
Lobothallia epiadelpha	Russia	Paukov 1881	MK347505	-	MK348232	Paukov et al. 2019
Lobothallia praeradiosa	Russia	Paukov AGP 20120606–12	MK347501	-	MK348229	Paukov et al. 2019
Lobothallia praeradiosa	**Russia**	**Paukov 3026**	MK347502	**PP832008**	**PP842140**	Paukov et al. 2019, **this paper**
Lobothallia subdiffracta	Russia	Frolov 178–1	MK347503	-	MK348233	Paukov et al. 2019
Megaspora verrucosa	Sweden	Nordin 6495	-	HM060725	HM060687	Nordin et al. 2010
Megaspora verrucosa	USA	St. Clair C54042	KC667053	KC667062	-	Sohrabi et al. 2013a
Ochrolechia balcanica	Greece	Schmitt (ESS-20968)	AF329172	AF329171	AF329170	Schmitt et al. 2001
Oxneriaria dendroplaca	Finland	Nordin 6366	HQ259260	HM060758	HM060720	Nordin et al. 2011
Oxneriaria dendroplaca	Sweden	Nordin 5952	HQ259259	HM060744	HM060706	Nordin et al. 2011
Oxneriaria mashiginensis	Sweden	Nordin 5790	EU057912	HM060732	HM060694	Nordin et al. 2007, 2010
Oxneriaria permutata	Sweden	Nordin 6027	EU057918	HM060747	HM060709	Nordin et al. 2007, 2010

Table 2 (continued)

Species	Country	Specimen number	GenBank number			Reference
			nc ITS	nc LSU	mt SSU	
Oxneriaria rivulicola	Sweden	Nordin 5957	EU057922	HM060753	HM060715	Nordin et al. 2007, 2010
Oxneriaria supertegens	Sweden	Nordin 6023	EU057938	HM060751	HM060713	Nordin et al. 2007, 2010
Oxneriaria supertegens	Norway	Owe-Larsson 9002	EU057936	HM060742	HM060704	Nordin et al. 2007, 2010
Oxneriaria verruculosa	Norway	Owe-Larsson 9007	EU057940	HM060741	HM060703	Nordin et al. 2007, 2010
Sagedia mastrucata	Sweden	Nordin 5481	EU057914	HM060737	HM060699	Nordin et al. 2007, 2010
Sagedia mastrucata	Norway	Nordin 5708	EU057913	HM060736	HM060698	Nordin et al. 2007, 2010
Sagedia zonata	Norway	Owe-Larsson 8942	EU057946	HM060738	HM060700	Nordin et al. 2007, 2010
Teuvoa junipericola	USA	Leavitt 742	JX306744	KC667054	-	Sohrabi et al. 2013a
Teuvoa junipericola	USA	Leavitt 767	JX306747	KC667055	-	Sohrabi et al. 2013a
Teuvoa uxoris	Spain	Rico & Pizarro 3622	JX306743	JX306757	-	Sohrabi et al. 2013a

Table 3 Summary of data partitions and substitution models used for phylogenetic inference

	ITS1	5.8S rRNA	ITS2	nuLSU	mtSSU
No. taxa	55	55	55	51	49
Position	1–167	168–338	339–461	462–906	907–1616
Substitution model (IQ-TREE)	TIM2 + F + G4	TNe + G4	TNe + G4	TIM + F + I + G4	TPM3u + F + I + G4
Substitution model (MrBayes)	GTR + G	K80 + I	GTR + G	GTR + I + G	HKY + I + G

externally similar *Lobothallia* is distinguished by the large, 22–36-µm-long ascospores and by the presence of substictic acid as a secondary metabolite. Its distinctness is supported by molecular phylogenetic analyses based on nuclear ITS, LSU, and mitochondrial SSU sequences.

Type species: *Atrostelia magnifica* Paukov, Davydov & Yakovchenko.

Russia, Republic of Tyva, Mongun-Taiginsky district, Mongun-taiga massif, right side of the Khairykan River valley at 4.5 km upstream from its mouth (Mugur River), talus within alpine meadows and mountain tundras, 50°18′01″ N, 90°11′42″ E, 2550 m asl., on rock, 11th of July 2014, leg. E. A. Davydov 21959 & L. S. Yakovchenko (LE–holotype; LE, ALTB, UFU–isotypes).

Description: thallus crustose, saxicolous, large, up to 7 cm in diameter, 1–2-mm thick, orbicular, radiate-placodioid, brown to dark-brown, covered by an epinecral layer that imparts tubercles of the surface a variegated pattern (Fig. 3a). Central parts of the thalli areolate. Areoles irregular, 1–4 mm in width and length or elongate, with wavy margins and tuberculate upper surface. Peripheral parts of thalli with radiate arranged furrows. Marginal areoles lobe-like elongate, 3–4 × 1–1.5 mm, often with finger-like blackish tips. Hypothallus absent. Cortical layer 25–50 µm, with cells 5–10 µm, covered by an epinecral layer 10–20 µm thick. The algal layer discontinuous, formed by vertically arranged groups of cells, 100–155 × 40–65 µm. Photobiont trebouxioid, cells 7–18 µm in diam., no algae below hypothecium.

Apothecia immersed, later appressed or sessile. Disc 0.5–1.5 mm, plane or weakly convex, black, weakly white-pruinose or without pruina, surrounded by a whitish in younger apothecia and later blackish thalline margin, concolorous with the disc (Fig. 3b). Hymenium 105–150 µm, paraphyses simple or scarcely branched, moniliform. Hypothecium 35–75 µm, bluish after treatment with an iodine solution. Outer exciple dark, 50–80 µm in its upper part, thinning to 20–25 µm under the hypothecium (Fig. 3c). Asci 8-spored; ascospores simple, arranged in two rows, hyaline, ovoid to ellipsoid, (22.0)23.6–[27.3]–31.0(36.0) × (12.0)13.4–[16.0]–18.6(23.0) µm; $n = 40$ (Fig. 3d).

Pycnidia frequent, immersed, 225–250 × 140–225 µm, with blackish mouth, simple or divided in several chambers. Conidia bacilliform, straight, (5.0)5.7–[6.8]–7.9(10.0) × 0.8–1 µm; $n = 80$.

Chemistry: substictic acid.

Etymology: The name of the genus refers to the dark radiate thalli of the type species. The species epithet refers to the large, eye-catching thalli easily noticeable in the field.

Habitat and distribution: *Atrostelia magnifica* is a common species on talus in alpine meadows and mountain tundras at elevations of 2400–2700 m. It is known only from the Mongun-Taiga massif.

Specimens examined: Russia, Republic of Tyva, Mongun-Taiginsky district, Mongun-taiga massif, left side of the Toolaity River valley at 3.5 km upstream from the

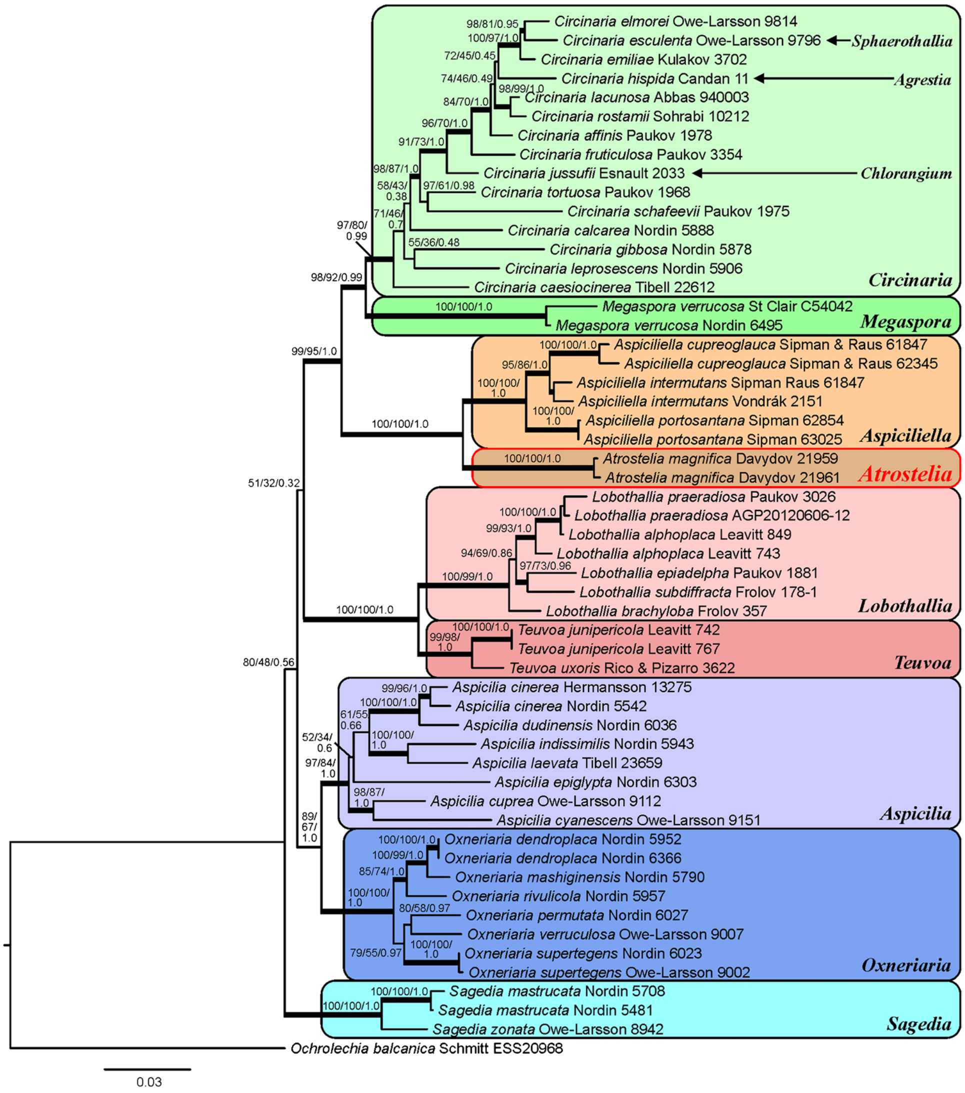

Fig. 2 Phylogenetic relationships within *Megasporaceae*, including the new genus *Atrostelia* (highlighted in red), derived from Bayesian inference of nuclear ribosomal ITS, LSU, and mtSSU sequence data. Values at tree branches indicate IQ-tree ML bootstrap percentages (left), RAxML bootstrap percentages (middle), and Bayesian inference with the Markov chain Monte Carlo (BMCMC) posterior probabilities (right). Thicker branches indicate when the bootstrap values of ML are > 0.70% and the BMCMC posterior probability is ≥ 0.95. *Ochrolechia balcanica* was used as an outgroup. The positions of the type species of *Agrestia*, *Chlorangium,*and *Sphaerothallia* in the tree are shown by arrows

Eski-Toolaity lake, mountain tundras, talus, 50°11′ N, 90°09′ E, 2450 m asl., on rock, 4th of July 2014, leg. E. A. Davydov 21956 & L. S. Yakovchenko (hb. Davydov & Yakovchenko); 50°10′47″ N, 90°08′50″ E, 2570 m asl., on rock, 4th of July 2014, leg. E. A. Davydov 21957 & L. S. Yakovchenko (hb. Davydov & Yakovchenko); 50°10′18″ N, 90°09′05″ E, 2670 m asl., on rock, 5th of July 2014, leg. E. A. Davydov 21958 & L. S. Yakovchenko (hb. Davydov & Yakovchenko, HMAS); 50°10′18″ N, 90°09′05″ E, 2670 m asl., on rock, 5th of July 2014, leg. E. A. Davydov 21960 & L. S. Yakovchenko (hb. Davydov & Yakovchenko); 50°18′01″ N, 90°11′42″ E, 2550 m asl., on rock, 11th of July 2014, leg. E. A. Davydov 21961 & L. S. Yakovchenko (hb. Davydov & Yakovchenko).

Comments: By their brown colour, thick epinecral layer and immersed apothecia in the central part of thalli, some samples of *Atrostelia magnifica* resemble *Aspiciliella cupreoglauca* (B. de Lesd.) Zakeri, Divakar & Otte but can be easily distinguished by the thick placodioid-lobate thallus and no colour change after treatment with KOH. Brown radiate thalli of *Atrostelia magnifica* are similar to *Lobothallia zogtii* Paukov & Davydov (Paukov et al. 2019), but the latter species differs in its convex apothecia, subhypothecial algal layer, smaller spores, and the presence of stictic acid. *Neoprotoparmelia capensis* V. J. Rico, A. Crespo & Garima Singh (Singh et al. 2018) is externally similar to the newly described species but has a distribution in South Africa, has sessile apothecia, smaller, oblong to fusiform spores, thick subhypothecial algal layer and α–alectoronic acid as a major secondary metabolite.

From the morphological point of view *Atrostelia* is a well circumscribed genus. It is similar to *Circinaria*, *Lobothallia* or *Oxneriaria* rather than to the genetically relative *Aspiciliella*. The overall number of morphological and anatomical characters (i.e., immersed young apothecia, spore and conidia size) that unite the putative genus with *Aspiciliella* is

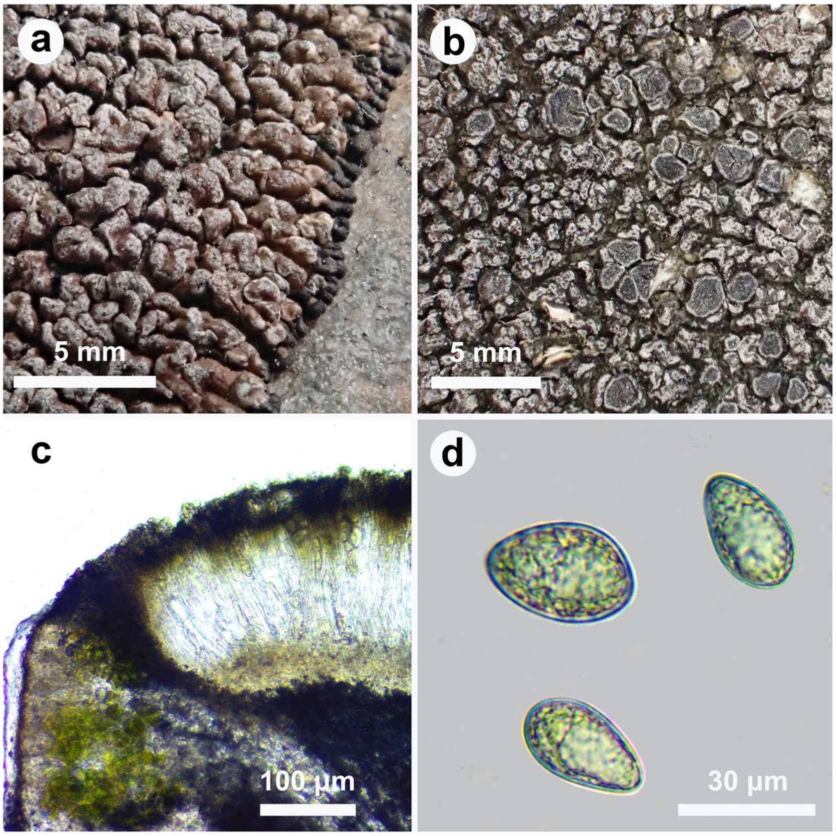

Fig. 3 Holotype of *Atrostelia magnifica*. **a** Part of the holotype showing peripheral lobes. **b** Central part of the thallus with apothecia. **c** Section of apothecium. **d** Ascospores

lower than the number of differences between them. Single-marker and concatenated phylograms suggest a well-supported but rather distant relationship between *Aspiciliella* and *Atrostelia* because the branch lengths of both taxa are longer than between the clades of accepted pairs of genera like *Lobothallia* and *Teuvoa* or *Aspicilia* and *Oxneriaria*. In order to maintain a uniform approach in the circumscription of genera in *Megasporaceae*, the suppose reasonable the separation of *Atrostelia* and *Aspiciliella*.

Discussion

The phylogeny of *Megasporaceae* assembled with the use of nu ITS, nu LSU, and mt SSU loci supported 9 genera in the family: *Aspicilia*, *Aspiciliella*, *Atrostelia*, *Circinaria*, *Lobothallia*, *Megaspora*, *Oxneriaria*, *Sagedia*, and *Teuvoa*. Three genera, *Agrestia*, *Chlorangium*, and *Sphaerothallia* are synonyms of *Circinaria*. Genera with large spores (*Aspiciliella*, *Atrostelia*, *Circinaria*, and *Megaspora*) form a supported clade in the phylogeny of the family, while the position of genera with smaller spores is not fully resolved, which necessitates the use of additional markers to achieve a more robust phylogeny in *Megasporaceae*.

We suppose that the large spores may be considered a plesiomorphic character in the family that unites large-spored *Megasporaceae* with other *Pertusariales*: *Pertusariaceae*, *Ochrolechiaceae*, and *Varicellariaceae*. In parallel with these families, the reduction of number of spores in asci occurs in *Circinaria*. Another character that unites this group of *Megasporaceae* with other *Pertusariales* is thin paraphyses in *Megaspora* similar to those in *Pertusariaceae*. The range of secondary metabolites in *Megasporaceae* is narrower than that in the other abovementioned *Pertusariales*. Two metabolites that, among others, could help in separation of genera in *Pertusariales* are orcinol depsides gyrophoric and lecanoric acid (Schmitt and Lumbsch 2004). The only known representative of *Megasporaceae* that contains lecanoric acid is *Circinaria schafeevii* (Tomin) Q. Ren (Ren and Zhang 2018) whose relationship with the family was supported here by molecular methods. It is a member of a large-spored branch, which, having a number of anatomical similarities revealed a chemical relatedness with other *Pertusariales* outside *Megasporaceae*.

The distribution of *Atrostelia magnifica* at the moment is restricted to the Mongun Taiga massif within the Altai mountain system where it is a dominant species in lichen communities at the northern slope of the mountain. It has a relatively isolated geographical position; however, several other Altai Mts. endemics, which were described recently from the talus in mountain tundras of Mongun Taiga, *Rhizocarpon smaragdulum* Davydov & Yakovchenko, *Sporastatia* *crassulata* Yakovchenko & Davydov, *Lecanora solaris* Yakovchenko & Davydov, and *Pulvinora stereothallina* Davydov & Yakovchenko, were also found on other mountain ranges in the South–East or Central Altai (Davydov and Yakovchenko 2017; Yakovchenko and Davydov 2018; Yakovchenko et al. 2019; Davydov et al. 2021). Similarly, we expect the range extension of the newly described species and genus in the neighbouring territories in China, Mongolia, Kazakhstan, and Russia.

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Data availability The sequences presented in this study are openly available in GenBank at https://www.ncbi.nlm.nih.gov/genbank/.

Declarations

Competing interests The authors declare no competing interests.

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