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BIODIVERSITY STUDIES

Notes on the genus *Trachyspora* (Phragmidiaceae, Pucciniales) in Russia based on morphological and molecular data

Vasily A. Dudka *

Vasily A. Dudka
e-mail: VDudka@binran.ru

Komarov Botanical Institute RAS,
St. Petersburg, Russia

* corresponding author

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ABSTRACT

This work provides information on the species of the genus *Trachyspora* in Russia. To date, two species have been identified: *Trachyspora alchemillae* s.l. and *T. melospora*. Based on nrITS and nrLSU sequence data, an investigation of eight specimens revealed for the first time that the previously known species *Trachyspora alchemillae* comprises two distinct phylogenetic lineages. However, no significant morphological differences were detected between these two clades; thus, we refrain from describing them as new taxa pending the examination of the type material. Detailed morphological descriptions based on light and scanning microscopy, supplemented by molecular data, are provided for all examined collections.

Keywords: *Alchemilla*, nrITS, nrLSU, plant disease, rust fungi, species diversity

РЕЗЮМЕ

Дудка А. В. Заметка о роде *Trachyspora* (Phragmidiaceae, Pucciniales) в России основанная на морфологических и молекулярных данных. Данная работа содержит сведения о видах рода *Trachyspora*, встречающихся на территории России. К настоящему времени идентифицировано два вида: *Trachyspora alchemillae* s.l. и *T. melospora*. На основе анализа нуклеотидных последовательностей ядерных рибосомальных генов nrITS и nrLSU по восьми образцам впервые установлено, что ранее известный вид *Trachyspora alchemillae* представляет собой совокупность двух филогенетически обособленных линий. Однако достоверных морфологических различий между указанными кладами выявлено не было, в связи с чем описание их в качестве новых таксонов не предпринимается вплоть до исследования типового материала. Для всех изученных коллекций приведены детальные морфологические описания, подготовленные с использованием световой и сканирующей микроскопии и дополненные молекулярными данными.

Ключевые слова: *Alchemilla*, nrITS, nrLSU, болезни растений, видовое разнообразие ржавчинные грибы

Trachyspora is a relatively small genus of rust fungi in the family Phragmidiaceae, Pucciniales (Aime & McTaggart 2021). Species in this genus are parasites exclusively on *Alchemilla* (Rosaceae) (Cummins & Hiratsuka 2003). All species are autoecious, microcyclic, infecting the leaves and forming aeciospores and unicellular teliospores on pedicels. The species of *Trachyspora* are distributed in Africa, Eurasia, and North America (Arthur 1934, Gäumann 1959, Boedijn 1959, Gjaerum & Cummins 1982).

Persoon (1796) described *Uredo alchemillae* Pers. on *Alchemilla vulgaris* L. Later. Later, Fuckel (1861) noted the presence of spherical, rough, multicellular teliospores in *Uredo alchemillae* and transferred this species to a separate genus, *Trachyspora*, as *Trachyspora alchemillae* (Pers.) Fuckel. Apparently, the description of teliospores in Fuckel's work as "vielzellig" and "multicellularia" should be considered an inaccuracy (Henderso 1973).

Previously, some authors did not recognize the genus *Trachyspora* and classified its representatives in the genus *Uromyces* based on the presence of unicellular teliospores (Fischer 1904, Grove 1913). However, other authors accepted the genus and noted its relationship with the genera *Gymnoconia* and *Phragmidium* (Arthur 1934, Tranzschel 1939, Gäumann 1959, Henderson 1973). Gäumann (1959) compared the genus *Trachyspora* with *Gymnoconia* based on the mechanism of teliospore pedicle separation from the hypha. Henderson (1973), while studying the features of the ultrastructural organization of spores, emphasized the similarity of the structure of *Trachyspora* teliospores with those of *Gymnoconia* and their affinity to the structure of *Phragmidium*, thereby confirming their relationship with each other. Tranzschel (1914) established a separate family, Gymnoconiaceae, for *Gymnoconia* and *Trachyspora* due to the distinctive features of their teliospores and their association with host plants from the Rosaceae. However, this proposal was not supported in subsequent studies. The first molecular studies on *Trachyspora* revealed its relationship to *Gymnoconia* and its placement within the Phragmidiaceae (Maier et al. 2003).

Currently, the genus comprises five species worldwide: *Trachyspora alchemillae*, *T. keniensis* Gjaerum & Cummins, *T. melospora* (Therry) Tranzschel, *T. pentaphylleae* Gäum. and *T. wurthii* (E. Fisch.) Dietel (Boedijn 1959, Gäumann 1959, Gjaerum & Cummins 1982). One more species, formerly belonging to the genus *Trachyspora*, *Trachyspora vestita* (Dietel) J.C. Lindq., occurring on *Sapium* (Euphorbiaceae), was revised and redescribed as the hyphomycete species *Chlamy-*

domyces palmarum (Cooke) E.W. Mason. (Cummins & Hiratsuka 2003).

Two species, *Trachyspora alchemillae* s.l. and *T. melospora*, are currently known from Russia (Tranzschel 1939). The possibility that *T. alchemillae* has a more heterogeneous intraspecific structure and may represent a species complex has long been noted by a number of authors (Tranzschel 1939, Gäumann 1959).

The aim of this study was to revise the species diversity of the genus *Trachyspora* in Russia based on modern collections and herbarium specimens using morphological and molecular approaches.

MATERIAL AND METHODS

Specimen sampling and morphological analyses

Nine specimens were examined in the study. The studied specimens were collected from the Arkhangelsk Oblast and Murmansk Oblast. Additionally, specimens from the Leningrad Oblast and the Republic of Dagestan were studied, which are preserved in the Herbarium of the Komarov Botanical Institute RAS (LE F).

The dried specimens were morphologically examined using light (LM) and scanning electron microscopy (SEM). Microscope photographs of affected plant sections were taken using a Stereo Microscope ZEISS Axio Zoom.V16 (Zeiss, Germany) equipped with ZEISS Axiocam 820 color digital camera and ZEN 3.10 software. To study the microstructure, hand-cut sections of affected leaf areas were mounted in Cotton Bule or Hoyer's mounting medium (Hoyer's solution) and examined under a light microscope Axio Scope A1 LED (Zeiss, Germany) equipped with ZEISS Axiocam 807 color digital camera and AxioVision SE64 software version 4.8.3.0. Measurements represent mean values plus/minus standard deviation and minimum and maximum values are given in parentheses. Microstructure measurement was carried out on the basis of electronic images using the Piximètre program version 5.10 R 1541 (Piximètre 2020).

To prepare samples for surface structure examination using SEM, small plant parts with aeciospores and teliospores were attached to aluminium stubs holders by double-adhesive tape, coated with gold and then observed with a JEOL JSM-6390LA Analytical Scanning Electron Microscope (USA).

The collected specimens were deposited in the Mycological Herbarium of the Komarov Botanical Institute (LE F).

DNA extraction and sequencing

DNA extraction procedure was fully consistent with the commercial protocol of the Phytosorb Kit (Syntol, Russia). For amplification and sequencing of the ITS1–5.8S–ITS2 and LSU nrDNA loci, pairs of oligonucleotide primers ITS1f-ITS4rust (Gardes & Bruns 1993, Beenken et al. 2012) and LRust1R-LR6 (Vilgalys & Hester 1990, Beenken et al. 2012) were used, respectively. Amplification was performed using standard PCR protocols for the primers used (White et al. 1990). The PCR products were then purified using the Cleanup Mini Gel Extraction Kit ("Evrogen", Moscow, Russia). Sequencing was performed with the ABI 3500 genetic analyzer (Applied Biosystems, California, USA). The raw data were edited and assembled in MEGA 11 (Tamura et al. 2021). All obtained nucleotide sequences were deposited in the GenBank with accession numbers as listed in Table 1.

All microscopic and molecular studies of specimens were carried out at the Center for collective use of scientific equipment "Cellular and molecular technology of studying plants and fungi" (Komarov Botanical Institute of the Russian Academy of Sciences, St. Petersburg).

Molecular phylogenetic analyses

For this study, eight nrITS and six nrLSU sequences of *Trachyspora* were newly generated. In addition, 20 nrITS sequences and 16 nrLSU sequences of taxa from the other genera of Phragmidiaceae (including sequences of *Kuehneola* as an outgroup) were retrieved from the GenBank database (https://www.ncbi.nlm.nih.gov/GenBank) for phylogenetic analyses (Table 1). The sequences of both loci were separately aligned using MAFFT 7 (Katoh et al. 2019, https://mafft. cbrc.jp/alignment/server/index.html) with the FFT-NS-i option and manually adjusted where necessary using MEGA 11.

Phylogenetic reconstructions were performed using Maximum Likelihood (ML) and Bayesian Inference (BI) analyses for combined nrITS+nrLSU dataset. Before the analyses, the best-fit substitution models were estimated separately for both alignments using FindModel web server (http://www. hiv. lanl. gov/content/sequence/findm odel/findmodel.htm) under the Akaike information criterion. The HKY+F+G4 model was chosen for the nrITS dataset and TN+F+G4 for the nrLSU. Hence, a partitioned evolution model for concatenated nrITS+nrLSU dataset in ML and Bayesian analyses to reconstruct a robust phylogenetic hypothesis was used.

Maximum likelihood analysis was run on IQ-TREE web server (Trifinopoulos et al. 2016) with 1000 rapid bootstrap replicates. BI analysis was performed with MrBayes 3.2.7 software (Ronquist et al. 2012), for two independent runs, each with 7 000 000 generations under described model and four chains with sampling every 100 generations. To check for convergence of MCMC analyses and to get estimates of the posterior distribution of parameter values, Tracer 1.7.1 was used (Rambaut et al. 2018). We accepted the result where the ESS (Effective Sample Size) was above 200 and the PSRF (Potential Scale Reduction Factor) was close to 1. Branches with bootstrap support (BS) and posterior probabilities (PP) values greater than or equal to 70 % and 0.90, respectively, were considered significantly supported (Hillis & Bull 1993). Tree topologies were then edited and visualized in FigTree (http://tree.bio.ed.ac.uk/).

Statistical analysis

Statistical evaluation of the structural measurement data was performed using the RStudio software environment, version 4.5.1 (R Core Team, 2025). Prior to conducting Welch's t-test, the data were tested for normality using the Shapiro-Wilk test.

Table 1. List of species used in the molecular analysis with host pants/substrate, collection information, GenBank accession numbers, and sources.

Species	Host/substrate	Country	Collection	Accession number of nrITS in GenBank NCBI	Accession number of nrLSU in GenBank NCBI
Gerwasia rubi-setchuenensise	*Rubus buergeri*	China	GMB4052	PQ472135	PQ456449
Gerwasia rubi-setchuenensise	*Rubus buergeri*	China	GMB4075	PQ472134	PQ456448
Gymnoconia peckiana	*Rubus* sp.	China	IBAR11161	MW729550	MW729478
Gymnoconia peckiana	*Rubus* sp.	—	—	GU058010	GU058010
Hamaspora rubi-alceifolii	*Rubus alceifolius*	China	GMB0109	OQ067094	OQ067532
Hamaspora rubi-alceifolii	*Rubus alceaefolius*	China	GMB0116	OQ067095	OQ067533
Kuehneola uredinis	*Rubus* sp.	USA	LD1029	GU058013	GU058013
K. uredinis	*Rubus* sp.	Mexico	CRM-3	OM009297	OM009297
Phragmidium potentillae	*Potentilla chinensis*	China	HMJAU8609	MK296538	MK296520
Phragmidium potentillae	*Potentilla chinensis*	China	BJFCR 00961	MN264720	MN264738
Phragmidium rosae-pimpinellifoliae	*Rosa sp.*	Russia	LE F-347563	PP621925	PP621904
Phragmidium rosae-pimpinellifoliae	*Rosa sp*	Russia	LE F-347564	PP621924	PP621903
Trachyspora alchemillae (as *T. intrusa*)	—	—	HMAS172049	MW729623	MW729545
Trachyspora alchemillae (as *T. intrusa*)	—	—	HMAS172318	MW729624	MW729546
Trachyspora alchemillae (as *T. intrusa*)	*Alchemilla vulgaris*	Switzerland	BPI 843828	DQ354550	DQ354550
Trachyspora alchemillae	***Alchemilla filicaulis***	**Russia**	**LE F-341676**	**PZ168459**	**—**
Trachyspora alchemillae	***Alchemilla glomerulans***	**Russia**	**LE F-341583**	**PZ168457**	**PZ164344**
Trachyspora alchemillae	***Alchemilla murbeckiana***	**Russia**	**LE F-353901**	**PZ168452**	**PZ164339**
Trachyspora alchemillae	***Alchemilla murbeckiana***	**Russia**	**LE F-353777**	**PZ168456**	**PZ164343**
Trachyspora alchemillae	***Alchemilla filicaulis***	**Russia**	**LE F-341699**	**PZ168458**	**—**
Trachyspora alchemillae	***Alchemilla murbeckiana***	**Russia**	**LE F-353902**	**PZ168453**	**PZ164340**
Trachyspora alchemillae	***Alchemilla hirsuticaulis***	**Russia**	**LE F-353903**	**PZ168454**	**PZ164341**
Trachyspora alchemillae	***Alchemilla hirsuticaulis***	**Russia**	**LE F-353904**	**PZ168455**	**PZ164342**
Triphragmium anomalum	*Filipendula palmata*	Russia	LE F-73827	PX636604	—
Triphragmium anomalum	*Filipendula palmata*	Russia	LE F-73850	PX636606	—
Triphragmium filipendulae	*Filipendula hexapetala*	Russia	LE F-75095	PX636608	—
Triphragmium filipendulae	*Filipendula vulgaris*	Russia	LE F-232405	PX636610	—
Triphragmium ulmariae	*Filipendula ulmaria*	Russia	LE 73814	PX636614	—
Triphragmium ulmariae	*Filipendula ulmaria*	Russia	LE 73792	PX636615	—

Note: Bold – new sequences generated for this paper; "—" – no data.

Terminology of rust fungi

In this work, an ontogenetic system for rust life cycles is used (Cummins & Hiratsuka 2003).

RESULTS

Phylogenetic analysis

The combined dataset of nrITS+nrLSU sequences contained 1469 characters, including gaps (ITS: 1–607 and LSU: 608–1469). Both Bayesian and Maximum likelihood analyses produced the same topology. Therefore, we present only the ML tree with both BS and PP val¬ues (Fig. 1). The four ITS sequences of *T. alchemillae* 'Clade 2' are 100 % identical to each other and 56 % distinguishable from *T. alchemillae* 'Clade 1'. Sequences from *T. alchemillae* 'Clade 1' are less identical to each other, ranging from 73–100 %. The nine LSU sequences of *T. alchemillae* s.l. were 99.9–100 % identical to each other.

In the phylogenetic analysis of two genetic markers, seven major clades were identified: *Gerwasia*, *Gymnoconia*, *Hamaspora*, *Kuehneola*, *Phragmidium*, *Trachyspora* and *Triphragmium*, in accordance with earlier studies (Aime & McTaggart 2021). In the resulting phylogenetic tree (Fig. 1), species of the genus *Trachyspora* clustered in a single monophyletic clade with the highest support (PP = 1, BS = 100 %). Based on the phylogenetic analysis, the species *Trachyspora alchemillae* forms two clades: *T. alchemillae* 'Clade 1' (PP = 0, BS = 74 %) and *T. alchemillae* 'Clade 2' (PP = 0.99, BS =92 %). Based on the phylogenetic data, we conclude that *T. alchemillae* is not genetically homogeneous.

Taxonomy

***Trachyspora alchemillae* (Pers.) Fuckel**, Bot. Ztg. 19(no. 35): 250 (1861) s.l. (≡*Uredo alchemillae* Pers., Observ. mycol. (Lipsiae) 1: 98 (1796); ≡*Uromyces alchemillae* (Pers.) G. Winter, Hedwigia 19(3): 35 (1880); =*Uredo intrusa* Grev., Fl. Edin.: 436 (1824); =*Uromyces intrusus* (Grev.) Lév., Annls Sci. Nat., Bot., sér. 3 8: 376 (1847); =*Trachyspora intrusa* (Grev.) Arthur, Manual of the Rusts in the United States & Canada: 97 (1934))

We consider *T. alchemillae* a complex species. Below are descriptions for each lineage according to their clade: *T. alchemillae* 'Clade 1' and *T. alchemillae* 'Clade 2'.

Trachyspora alchemillae 'Clade 1'

Description: Spermogonia not observed. Aecia subepidermal, erumpent, without peridium, covered by a piece of the torn epidermis (Fig. 3A), hypophyllous, almost covering the lower leaf surface, often coalescing, seldom solitary sori on the upper surface of the leaf, pulverulent, bright orange when fresh condition (Fig. 2A), colorless in herbarium (Fig. 2B). Aeciospores single on pedicels, globose, obovate, ellipsoid, (16.5–)19.0–23.0(–25.0) × (13.5–)16.0–19.0(–21.0) μm (M=21.40×19.31, N=250) (Fig. 4A–B), bright orange when fresh, colorless in herbarium, thin-walled, 0.5–1 μm (N=50), hyaline, densely echinulate (under SEM, individual spines-like often merge at the base all the way up to the top forming ridges, Fig. 5A–B), with conspicuous place of attachment aeciospore pedicel (Fig. 5D), germ pores not observed, pedicels hyaline, thin-walled, rarely observed. Uredinia not observed. Telia replacing the aecia (Fig. 2B), subepidermal, erumpent, without peridium, covered by a piece of the torn epidermis, hypophyllous, almost covering the lower leaf surface, often coalescing, seldom solitary sori on the upper surface of the leaf, pulverulent, from light brown to dark brown colour (Fig. 2B),

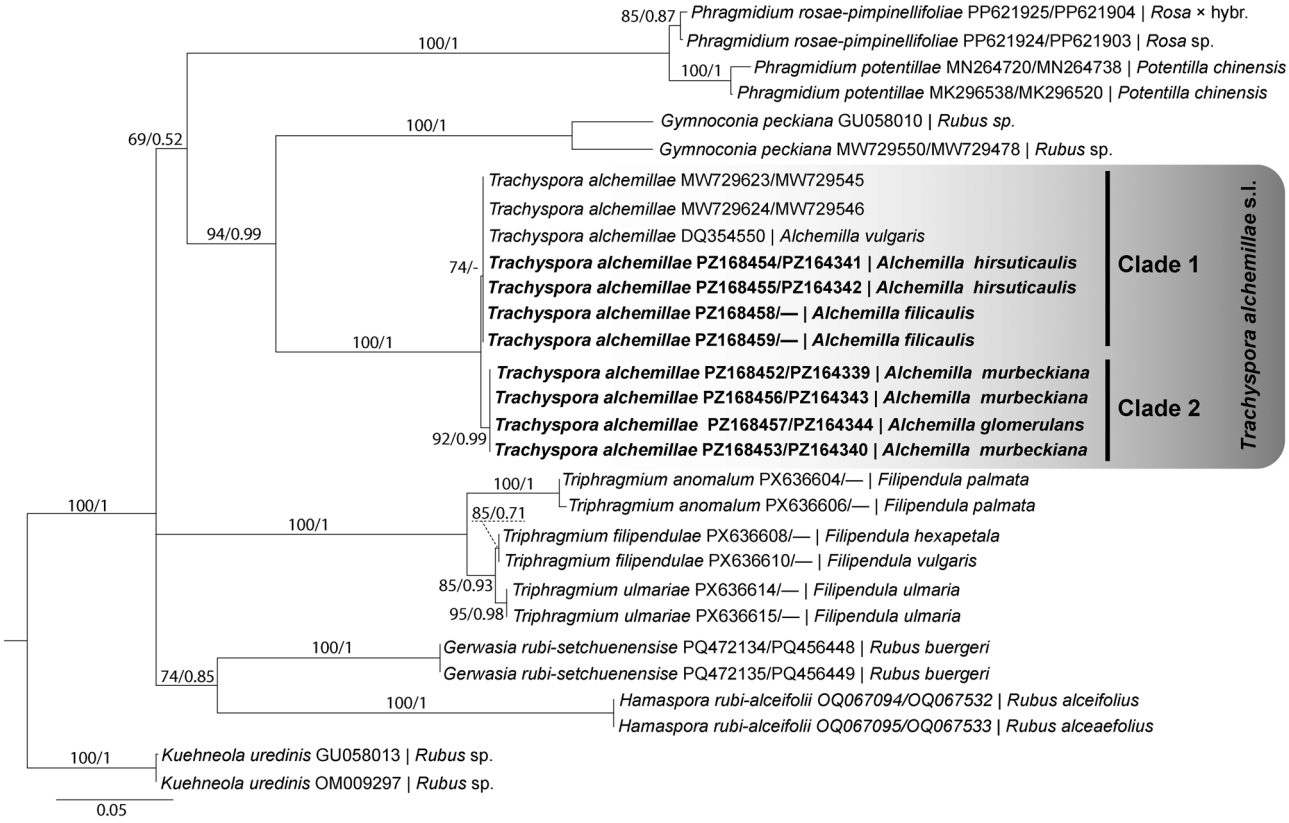

Figure 1 Phylogenetic tree from the ML analysis of the nrITS+nrLSU dataset for Phragmidiaceae species, with *Kuehneola uredinis* as outgroup. Bootstrap support values and Posterior probability (BS/PP) are given above the branches. All tips are labelled with taxon name, GenBank accession numbers, host and country. For taxa highlighted in bold, sequences are generated in present study

later producing localised telia, 250–430 μm in diam, subepidermal, erumpent, without peridium, covered by a piece of the torn epidermis, on the lower leaf surface, pulverulent, brown colour (Fig. 2C, Fig. 3B–C). Teliospores single on pedicels, irregularly, globular, ellipsoid, (23.5–)25.5–32.0(–36.0) × (18.5–)21.0–26.0(–27.5) μm (M=28.95×23.49, N=250) (Fig. 4C–D), from light brown to brown color, thick-walled, two-layer, 2.5–3.5 μm (N=50), inner layer 1.2–1.5(–2) (N=50) μm, brown, from smooth to verrucose (mostly at the top) (Fig. 5E–F), germ pores not observed, pedicels hyaline, thin-walled, (6.4–)7.1–9.7(–10.7) μm (M=8.17, N=50) wide.

Host: *Alchemilla filicaulis* Buser and *A. hirsuticaulis* H. Lindb.

Specimens examined: Russia, St. Petersburg, Dudergof station, on A. filicaulis, coll. Kondrakova, 25.06.1948, LE F-341676 (GenBank PZ168459 – ITS); Leningrad Oblast, Priozersk District, on *A. filicaulis*, coll. Pavlova, 25.07.1966, LE F-341699 (GenBank PZ168458 – ITS); Arkhangelsk Oblast, Kholmogorsky District, nearby of Ust-Pinega, Pechka River, 64.13101°N 41.9258°E, 10 m, on *A. hirsuticaulis*, coll. V.A. Dudka, 11.06.2025, LE F-353903 (GenBank PZ168454 – ITS, PZ164341 – LSU); ibid, nearby of Pechki, Pechka River, 64.13140°N 41.97759°E, 15 m, on *A. hirsuticaulis*, coll. V.A. Dudka, 12.06.2025, LE F-353904 (GenBank PZ168455 – ITS, PZ164342 – LSU).

Note: The shape and size of the aeciospores and teliospores are almost identical in *Trachyspora alchemillae* 'Clade 1' and *T. alchemillae* 'Clade 2'. The base of the teliospore pedicel in *T. alchemillae* 'Clade 1' is slightly narrower than in *T. alchemillae* 'Clade 2'.

Trachyspora alchemillae 'Clade 2'

Description: Spermogonia not observed. Aecia subepidermal, erumpent, without peridium, covered by a piece of the torn epidermis (Fig. 3D), hypophyllous, almost covering the lower leaf surface, often coalescing, seldom solitary sori on the upper surface of the leaf, pulverulent, bright orange when fresh condition (Fig. 2D), colorless in herbarium. Aeciospores single on pedicels, globose, obovate, ellipsoid, (17.5–)18.5–22.5(–25.5) × (15–)17.0–19.5(–21.0) μm (M=21.43×19.33 μm, N=250) (Fig. 4E–F), bright orange when fresh, colorless in herbarium, thin-walled, 0.5–1 μm (N=50), hyaline, densely echinulate (under SEM, individual spines-like often merge at the base all the way up to the top forming ridges, Fig. 6A–B), with conspicuous place of attachment aeciospore pedicel (Fig. 5C), germ pores not observed, pedicels hyaline, thin-walled, rarely observed. Uredinia not observed. Telia replacing the aecia (Fig. 2E), subepidermal, erumpent, without peridium, covered by a piece of the torn epidermis (Fig. 2F), hypophyllous, almost covering the lower leaf surface, often coalescing, seldom solitary sori on the upper surface of the leaf, pulverulent, from light brown to dark brown colour (Fig. 2F–H). Teliospores single on pedicels, irregularly, subglobose, ellipsoid, (23.5–)25.5–31.5(–36.0) × (16.5–)20.0–25.5(–27.5) μm (M=28.71×22.97 μm, N=250) (Fig. 4G–H), from light brown to brown colour, thick-walled, two-layer, 2.5–3.5 μm (N=50), inner layer 1.2–2.0 μm (N=50), brown, from smooth to verrucose (mostly at the top) (Fig. 6D–F), germ pores not observed, pedicels hyaline, thin-walled, (6.7–)7.3–10.8(–14.2) μm (M=9.31, N=50) wide.

Host: *Alchemilla glomerulans* Buser and *A. murbeckiana* Buser.

Specimens examined: Russia, Murmansk Oblast, Kola District, Serebryanskaya Road, 68.85313°N 34.83654°E, 240 m, on *A. glomerulans*, coll. V.A. Dudka, 26.06.2023, LE F-341583 (GenBank PZ168457 – ITS, PZ164344 – LSU); ibid, Sredniy Peninsula, 69.78596°N 31.95899°E, -12 m, on *A. murbeckiana*, coll. V.A. Dudka, 28.06.2023, LE

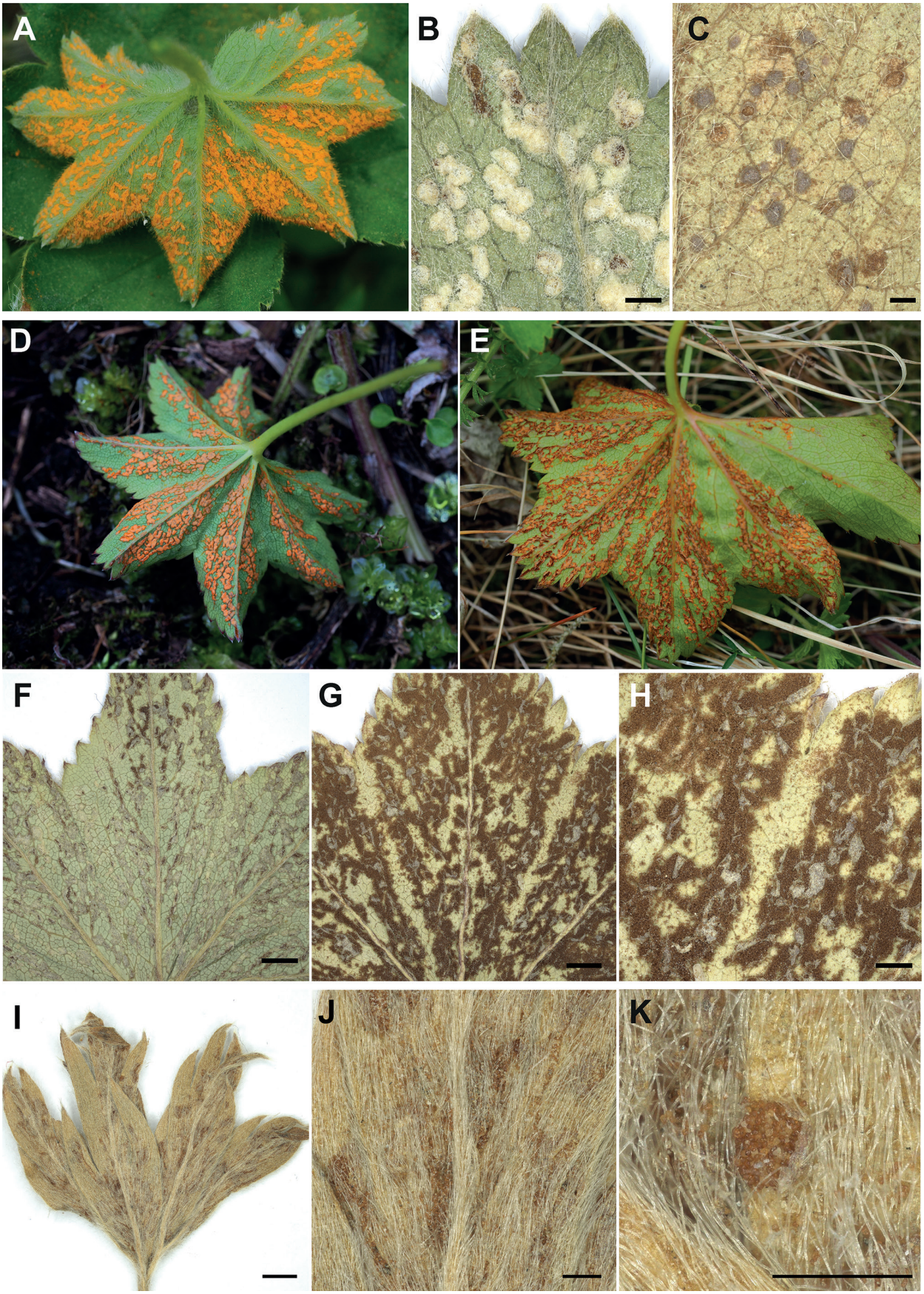

Figure 2 *Trachyspora* sp. on *Alchemilla* sp. *Trachyspora alchemillae* 'Clade 1': A – Aecia on leaf surface *Alchemilla hirsuticaulis* (LE F-353903), B – Aecia and telia on leaf surface *A. hirsuticaulis* (LE F-353903); C – Telia on leaf surface *A. filicaulis* (LE F-341676); *Trachyspora alchemillae* 'Clade 2': D – Aecia on leaf surface *Alchemilla murbeckiana* (LE F-353902), E – Aecia and telia on leaf surface *A. murbeckiana* (LE F-35377); F–H – Telia on leaf surface *A. murbeckiana* (LE F-353901); *Trachyspora melospora* on *Alchemilla sericea* (LE F-70290): I, J – Aecia on leaf surface; K – Aecium on leaf surface. Scale bar: B – 0.5 mm, C – 2 mm, F-G – 2 mm, H – 1 mm, I – 2 mm, J-K – 0.5 mm

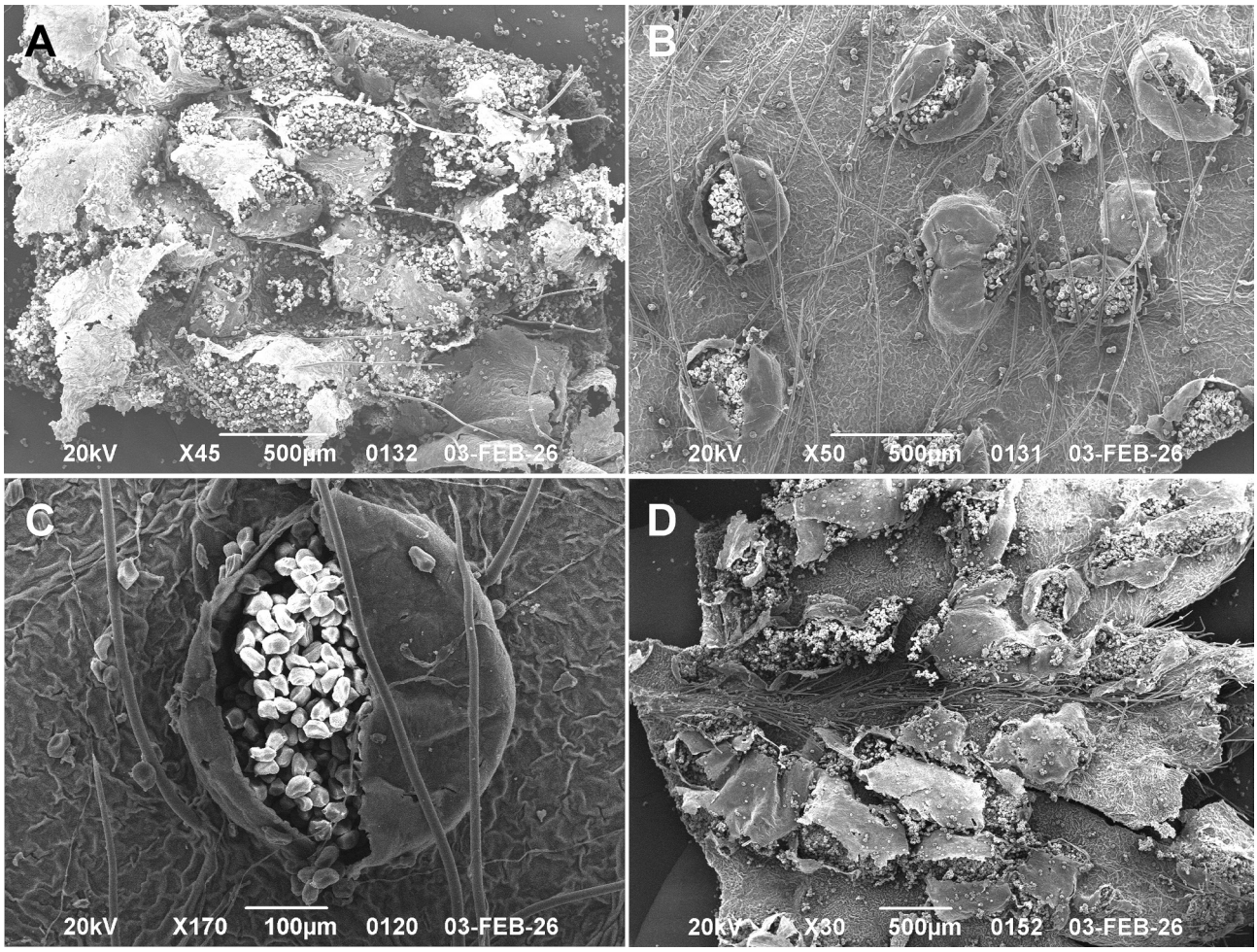

Figure 3 *Trachyspora* sp. on *Alchemilla* sp. (SEM). *Trachyspora alchemillae* 'Clade 1': A – Aecia on leaf surface *Alchemilla hirsuticaulis* (LE F-353903), B – Telia on leaf surface *A. filicaulis* (LE F-341676), C – Telium on leaf surface *A. filicaulis* (LE F-341676); *Trachyspora alchemillae* 'Clade 2': D – Aecia on leaf surface (LE F-35377)

F-353777 (GenBank PZ168456 – ITS, PZ164343 – LSU); ibid, Pechenga District, Rybachy Peninsula, Syuvya River, 69.82771°N 32.02386°E, 27 m, on *A. murbeckiana*, coll. V.A. Dudka, 29.06.2023, LE F-353901 (GenBank PZ168452 – ITS, PZ164339 – LSU); ibid, Zubovka, 69.81066°N 32.25684°E, 15 m, on *A. murbeckiana*, coll. V.A. Dudka, 30.06.2023, LE F-353902 (GenBank PZ168453 – ITS, PZ164340 – LSU).

Notes: All specimens belonging to *Trachyspora alchemillae* 'Clade 2' were found in the tundra zone. It is not known for certain whether members of this clade are capable of forming local telia patches, as *T. alchemillae* 'Clade 1' upon reinfection. No such infection was observed in our study.

***Trachyspora melospora* (Therry) Tranzschel**, in Elenkin, Kamchatskaya Ekspeditsiya [Kamchatkan Expedition], Bot. Otd. 2 Sporovyya rasteniya Kamchatki [Spore-bearing plants of Kamchatka]: 567 (1914) (≡*Uredo melospora* Therry, Annls Soc. bot. Lyon 2: 150 (1874) [1873-74]; ≡*Uromyces melosporus* (Therry) Syd. & P. Syd., Annls mycol. 4(6): 484 (1907); ≡*Trachysporella melospora* (Therry) Syd., Annls mycol. 19(3-4): 168 (1921); ≡*Trachyspora melospora* (Therry) Dietel, Annls mycol. 21(1/2): 88 (1923) nom. superfl.; =*Uromyces alchemillae-alpinae* E. Fisch., Bull. Soc. Bot. France 41: CCXLI (1895))

Description: Spermogonia and aecia not observed. Aeciospores single on pedicels, subglobose, ellipsoid, (19.0–)21.0–26.0(–27.0) × (16.5–)17.5–22.0(–25.0) µm (N=50) (Fig. 4I–J), bright orange when fresh, colorless in herbarium, thin-walled, 0.7–1 µm (N=50), hyaline, densely echinulate (under SEM, individual spines-like often merge at the base all the way up to the top forming ridges, Fig. 7B), with conspicuous place of attachment aeciospore pedicel (Fig. 4I), germ pores and pedicels not observed. Uredinia not observed. Telia replacing the aecia (?), subepidermal, erumpent, without peridium, covered by a piece of the torn epidermis (Fig. 7A), hypophyllous, almost covering the lower leaf surface, often coalescing, seldom solitary sori on the upper surface of the leaf, pulverulent, brown color (Fig. 2I–K). Teliospores single on pedicels, globose, subglobose, ellipsoid, (26.5–)27.5–33.5(–36.0) × (21.5–)23.0–27.5(–29.0) µm (N=50) (Fig. 4K–L), brown colour, thick-walled, two-layer, (2.5–)3.0–4.0 µm (N=50), inner layer (1.3–)1.5–2(–2.5) (N=50) µm, brown, stronly verrucose (Fig. 7C-D), germ pores not observed, pedicels hyaline, thin-walled, 7.5–10.0 µm wide.

Host: *Alchemilla sericea* Willd.

Specimens examined: Russia: Caucasus, Republic of Dagestan, the source of the Ilan-khevi river [Caucasus orient., Dagestania, superior montosa ad fl. Ilan-chewi], on *A. sericea*, Ruprecht J.F.,17.08.1860 (LE F-70290).

Note: *Trachyspora melospora* differs from *T. alchemillae* s.l. in its larger teliospores having strongly verrucose surface (Fig. 7C–D). *Trachyspora melospora* is also distinguished by its host range, which has been confirmed by infection experiments (Fischer 1903, 1915). This species is found in Europe

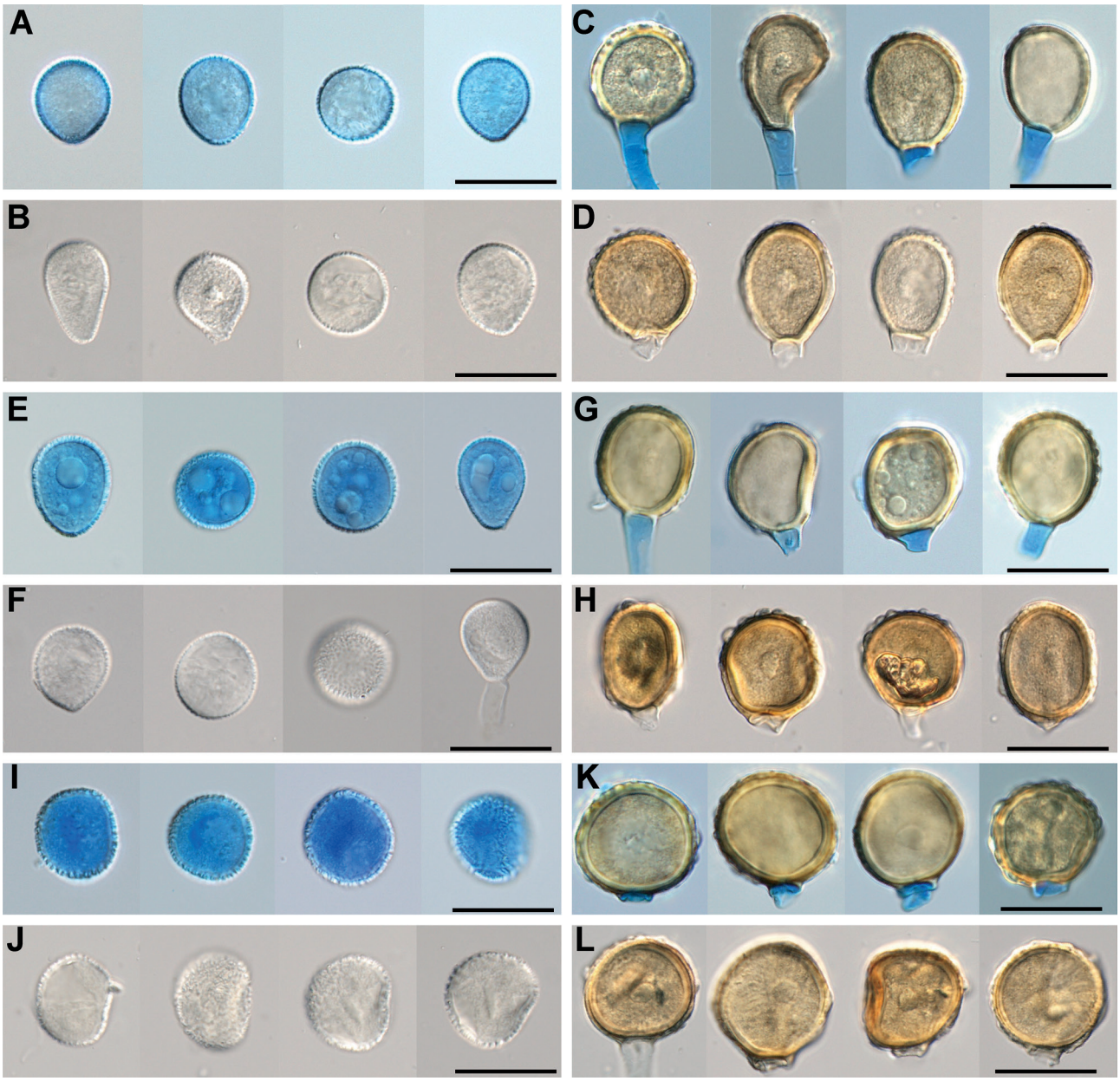

Figure 4 Aeciospores and teliospores *Trachyspora* sp. (LM). *Trachyspora alchemillae* 'Clade 1' (LE F-353903): A – aeciospores (Cotton blue), B – aeciospores (Hoyer's solution), C – teliospores (Cotton blue), D – teliospores (Hoyer's solution); *T. alchemillae* 'Clade 2' (LE F-353777): E – aeciospores (Cotton blue), F – aeciospores (Hoyer's solution), G – teliospores (Cotton blue), H – teliospores (Hoyer's solution); *T. melospora* (LE F-353777): I – aeciospores (Cotton blue), J – aeciospores (Hoyer's solution), K – teliospores (Cotton blue), L – teliospores (Hoyer's solution). Scale bar – 25 µm

on the species *Alchemilla alpina* L., *A. hoppeana* (Reichenb.) Dalla Torre, *A. indivisa* (Buser) Rothm., and *A. plicatula* Gand (Helfer 2005, Zwetko et al. 2024). Helfer (2005) originally described *Trachyspora pentaphylleae* as a variety of *T. melospora* var. *pentaphylleae* (Gäum.) S. Helfer on *Alchemilla pentaphylla* L. It was not possible to obtain *Trachyspora melospora* DNA sequences from our specimen LE F-70290.

DISCUSSION

Currently, no revision of the species of the genus *Trachyspora* has been carried out at the modern taxonomic level. All data on *Trachyspora* are presented only within the framework of comprehensive reviews (Petrova & Denchev 2004, Helfer 2005, Zwetko et al. 2024). However, as the results of our study have shown, the genus *Trachyspora* deserves special attention in its intraspecific taxonomy.

This study provides a review of the genus *Trachyspora* in Russia, using for the first time integrative taxonomic methods including light microscopy, scanning electron microscopy and molecular data for intraspecific taxonomy. Currently, two species of the genus are found in Russia. Our study revealed that *T. alchemillae* comprises at least two phylogenetic lineages, as determined by nrITS and nrLSU phylogeny (Fig. 1). However, we were unable to identify any reliable morphological differences between *T. alchemillae* 'Clade 1' and 'Clade 2'. It is worth noting that the application of Welch's two-sample t-test revealed statistically significant dif-

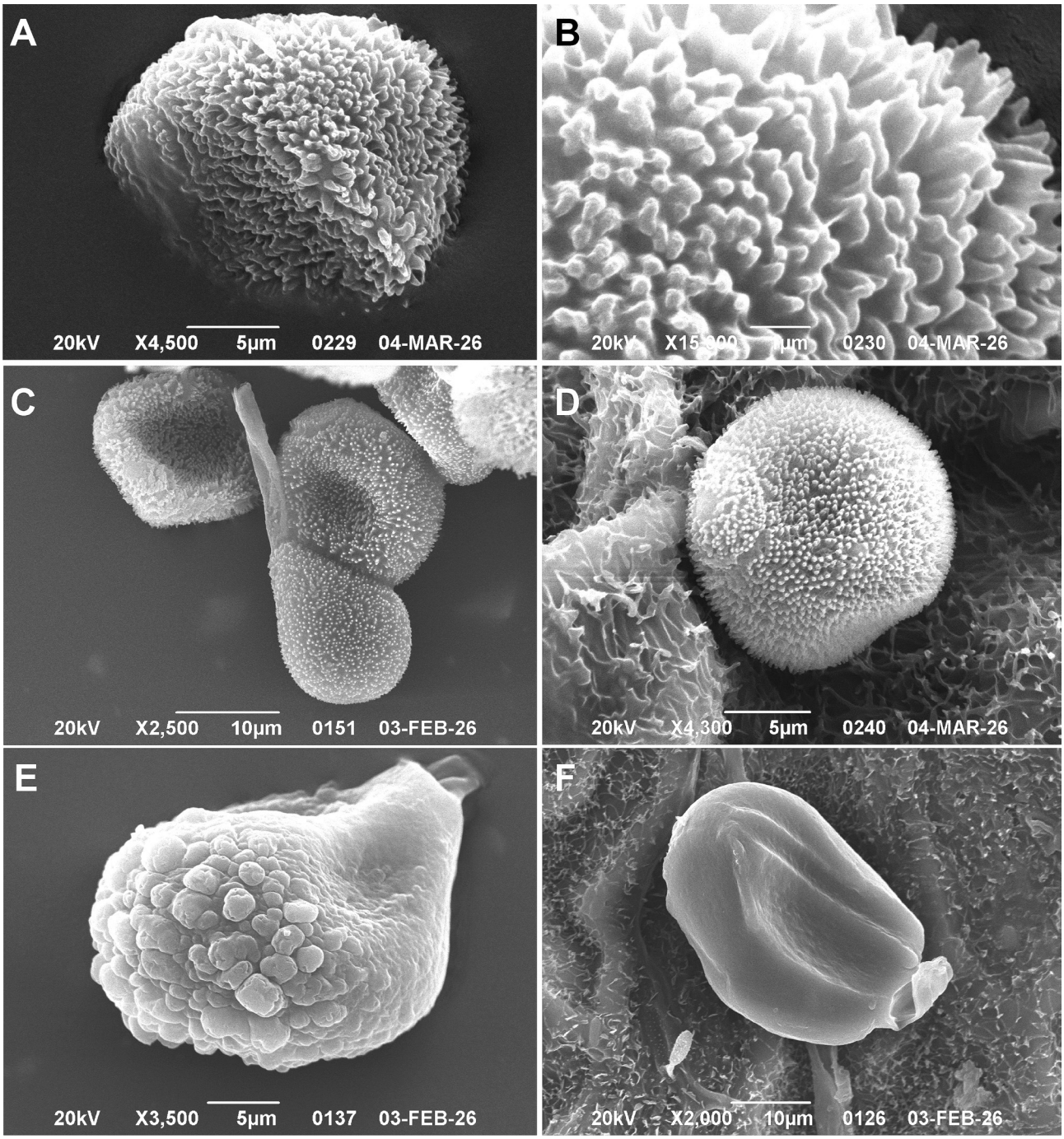

Figure 5 Aeciospores and teliospores of *Trachyspora alchemillae* 'Clade 1' (SEM): A – aeciospore (LE F-353903), B – surface of aeciospore (LE F-353903), C – aeciospore with pedicel (LE F-353903), D – place of attachment aeciospore pedicel (LE F-353904), E – verrucose surface of the teliospore (LE F-353903), F – even surface of the teliospore (LE F-353904)

ferences in the mean width between the *T. alchemillae* 'Clade 2' (M = 22.98) and *T. alchemillae* 'Clade 1' (M = 23.49) groups, ($p = 0.0099$). *Trachyspora alchemillae* 'Clade 1' samples have a significantly larger width compared to the *T. alchemillae* 'Clade 2' group. Additionally, the width of the pedicle cell base on the teliospores of *T. alchemillae* 'Clade 1' (M = 8.17 µm) is narrower than that of *T. alchemillae* 'Clade 2' (M = 9.32 µm). The application of the two-sample Welch t-test ($p < 0.001$) also revealed statistically highly significant differences in the average width of the pedicel between the groups. No statistical differences were found in the measurements of aeciospores and their wall, as well as in the length and thickness of the cell wall of teliospores. It is important to note that there is a significant variability in microstructure in terms of shape, size, and color in specimens of both *T. alchemillae* 'Clade 1' and *T. alchemillae* 'Clade 2'. This variability is observed both within a single sori and between different specimens. It was also noted that the time at which specimens are collected affects microstructural characteristics. For example, teliospores in young sori are smaller, the cell wall is paler, and the ornamentation is almost smooth with only slight verrucosity.

In our study, no telial symptoms in the form of localised spots were detected in *T. alchemillae* 'Clade 2'. The two specimens (LE F-341699 and LE F-341676) included in

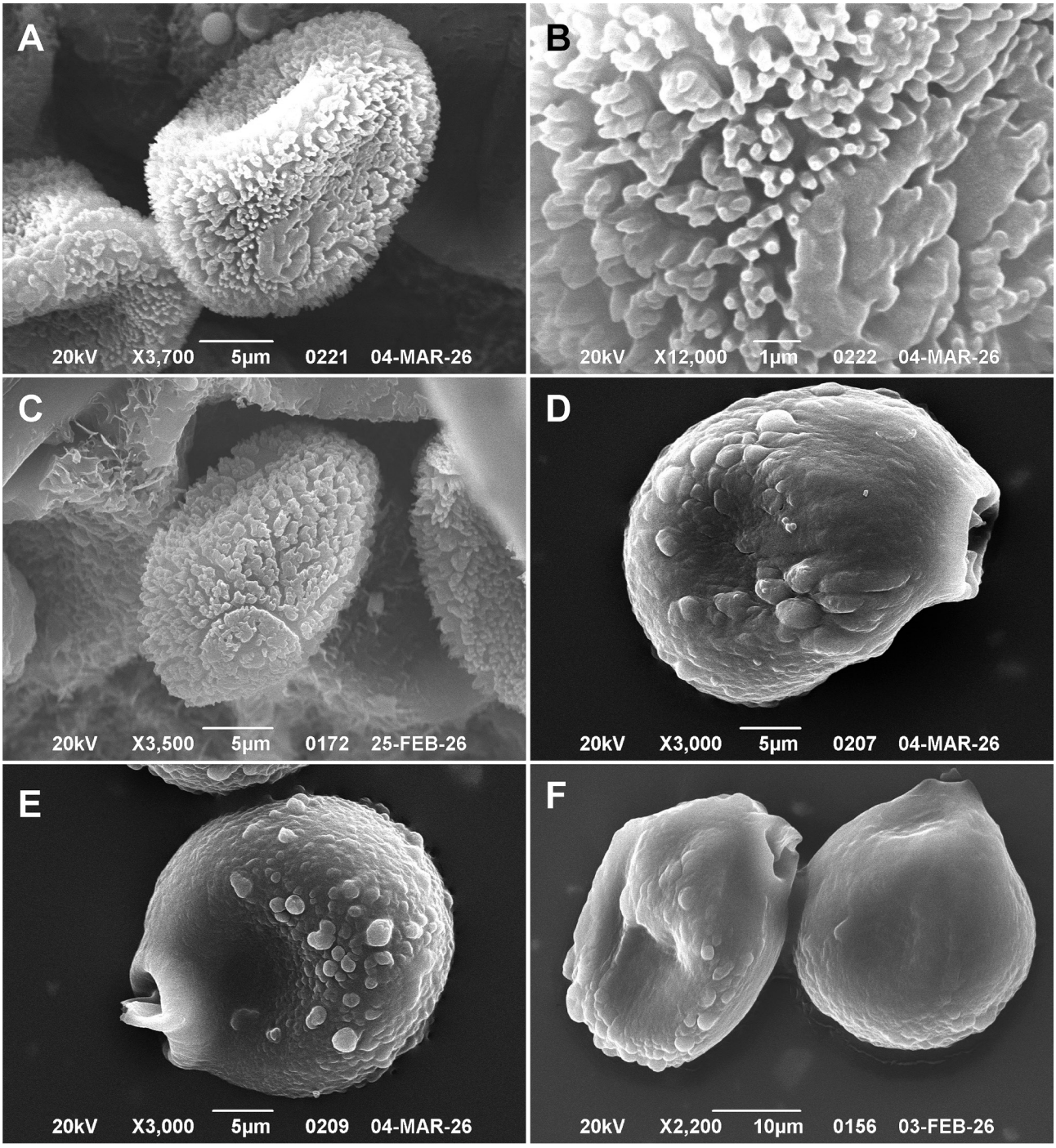

Figure 6 Aeciospores and teliospores of *Trachyspora alchemillae* 'Clade 2' (SEM): A – aeciospore (LE F-353901), B – surface of aeciospore (LE F-353901), C – place of attachment aeciospore pedicel (LE F-341655), D–E – verrucose surface of the teliospore (LE F-353902), F – verrucose and even surface of the teliospores (LE F-353777)

the analysis that exhibited localised telial symptoms both belonged to *T. alchemillae* 'Clade 1'. Including more specimens with local telial spots in the study may help to determine whether *T. alchemillae* 'Clade 2' forms them or not.

It is important to note that a significant number of nucleotide sequence differences of two clades among the specimens included in the phylogenetic analysis were found in the ITS1 region (4 bp), with a smaller number found in the ITS2 region (2 bp). Substitutions were also found in the nrLSU region (1 bp). *Trachyspora alchemillae* 'Clade 1' has low bootstrap support (BS = 74 %) and no posterior probability support (PP = 0) in the ML/BA analyses, respectively. This is likely due to the disparity in ITS sequence identity among the specimens within *T. alchemillae* 'Clade 1'.

The two clades differ in the host-plant species for *Trachyspora* specimens. The studied collections of *T. alchemillae* 'Clade 1' were found on *Alchemilla filicaulis* Buser and *A. hirsuticaulis* H. Lindb., while collections of *T. alchemillae* 'Clade 2' were found on *A. glomerulans* Buser and *A. murbeckiana* Buser. All of these host species had previously been identified as hosts for *T. alchemillae* s.l., except for on *A. hirsuticaulis*. It is unclear whether *T. alchemillae* s.l. is strictly specialised

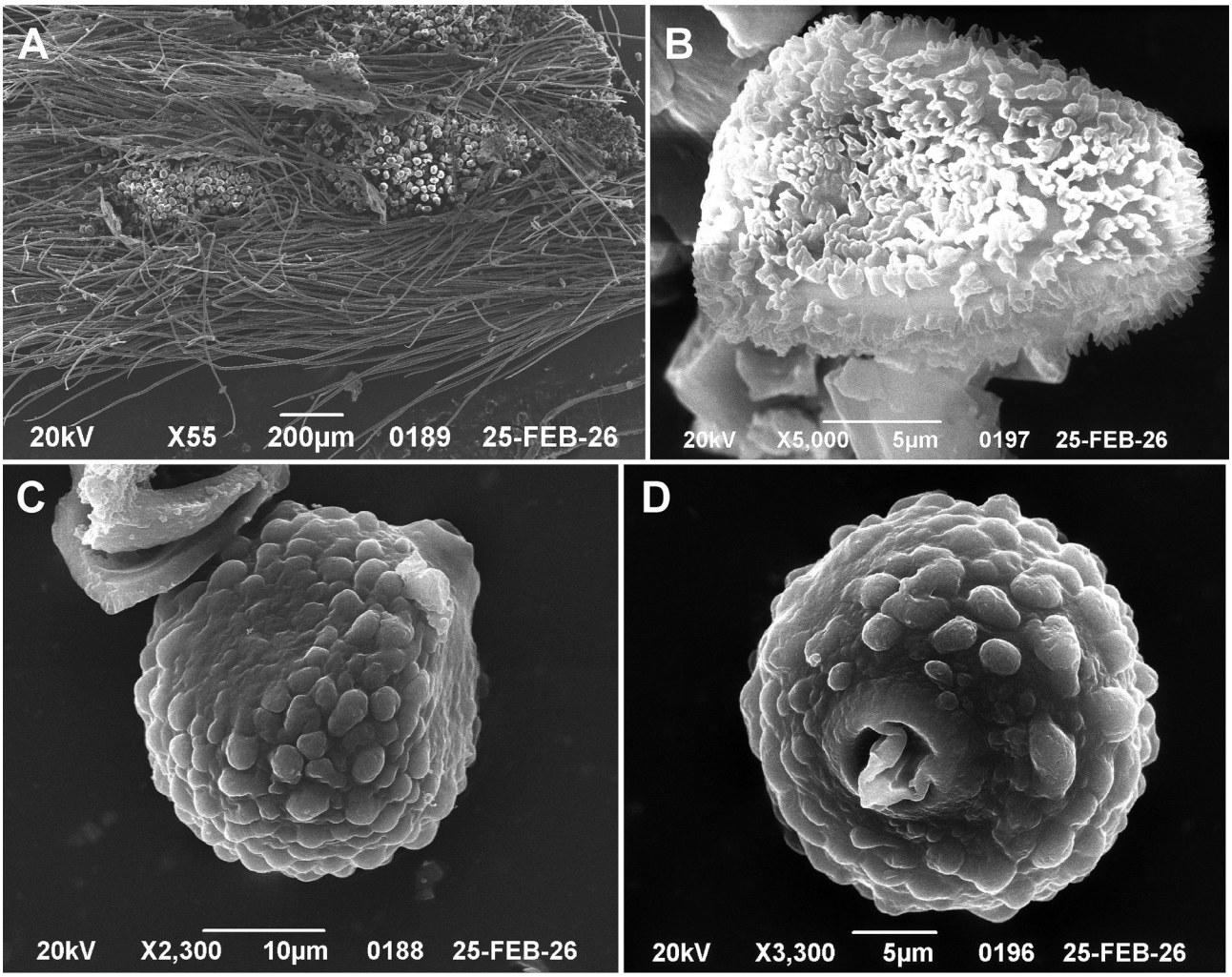

Figure 7 Aeciospores and teliospores of *Trachyspora melospora* (SEM) (LE F-70290): A – aecia on leaf surface on *Alchemilla sericea*; B – aeciospore; C-D – verrucose surface of the teliospore

to *Alchemilla* micro-species, but this seems unlikely to us. Nevertheless, further research is needed to shed light on the specialisation of *Trachyspora* within the *Alchemilla vulgaris* s.l. species complex. This requires the precise identification of *Alchemilla* species, which will involve botanists in the study in order to reliably determine the host.

The ecological characteristics of these clades should also be clarified. The four specimens included in the study, belonging to *Trachyspora alchemillae* 'Clade 2', were collected in the tundra. All specimens included in the study, belonging to the *T. alchemillae* 'Clade 1', were collected in the taiga. However, we do not know the exact ecological and host specialisation of the specimens included in the phylogenetic analysis from GeneBank. To address this, a greater number of observations using molecular methods must be incorporated.

In order to determine which specimens belong to *T. alchemillae* s. str., the type material must be examined in detail. A detailed description of its morphological characteristics and identification of its host are needed, as well as information on the ecological conditions under which the material was collected. Obtaining genetic material appears necessary, though this may be difficult given the age of the specimen. For this purpose, a high-quality epitypification study should be conducted.

In view of the above, and in order to avoid complicating future research, we have decided not to describe the species within the genus without further data and a thorough examination of the type material. At this point, our goal was only to identify a range of problems and tasks, the solution of which could lead to a clear understanding of the intraspecific problem of the *Trachyspora alchemillae* complex.

Trachyspora melospora is likely widespread in the Caucasus and may be found on other hosts of *Alchemilla* sect. *Alpinae* and sect. *Glaciales* this region. Molecular data on this species is also needed to determine whether *Trachyspora melospora* is homogeneous.

CONCLUSIONS

The genus *Trachyspora* is represented in Russia by two species: *Trachyspora alchemillae* s.l. and *T. melospora*. *Trachyspora alchemillae* s.l. comprises at least two distinct phylogenetic lineages. The individual lineages within *Trachyspora alchemillae* s.l. are morphologically nearly indistinguishable, except for the mean values of teliospore width and teliospore pedicel width, which highlights their cryptic diversity. This phenomenon may be attributed to the apomixis of the host plants

and convergent evolution. Examination of the *Trachyspora alchemillae* type material is required for comprehensive morphological and phylogenetic analyses, although this may be challenging due to the age of the original material. Further expansion of the dataset is necessary, including broadening the geographical sampling, incorporating alternative host plant species to elucidate host specialization, and analyzing additional loci (e.g., SSU, *RPB2*, *TEF1*, *COX3*, etc.). Resampling of *T. melospora* within Russia (the Caucasus) is required to facilitate a detailed investigation based on fresh material.

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