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TAXONOMIC REVISIONS

Additions to *Trichia botrytis* complex (Myxomycetes): 9 new species

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ABSTRACT

A taxonomic revision of the *Trichia botrytis* species complex based on the study of specimens from the LE and MYX fungaria was made. Six of 7 previously described species of the complex (except for *T. subfusca*) and 10 taxa new to science (9 species and 1 variety) were recognized. Each studied morphotype, including the previously known ones, is described in detail and illustrated with LM and SEM photographs. In addition, nrSSU, mtSSU, and EF1α genes sequences are given for all 16 morphotypes. The variability of different features (including fruiting body morphology and species ecology) is discussed thoroughly, as well as their taxonomic significance and applicability in practice. A set of features that are highly recommended to be used in subsequent descriptions of new *Trichia* species and closely related genera is proposed and some methodological recommendations for morphological analysis are given.

Keywords: Myxomycetes, Trichiales, multigene phylogeny, SEM, species complex, taxonomic revision

РЕЗЮМЕ

Бортников Ф.М., Бортникова Н.А., Гмошинский В.И., Приходько И.С., Новожилов Ю.К. Расширение комплекса *Trichia botrytis* (Myxomycetes): 9 новых видов. На основании исследования образцов из фунгариев LE и MYX проведена таксономическая ревизия комплекса *T. botrytis*. Рассмотрены 6 из 7 ранее описанных видов (за исключением *T. subfusca*) и описано 10 новых таксонов (9 видов и 1 разновидность). Каждый исследованный морфотип, включая ранее известные, детально описан и проиллюстрирован микрофотографиями, полученными с помощью светового и сканирующего электронного микроскопов. Кроме того, приводятся частичные последовательности генов nrSSU, mtSSU и EF1α для всех 16 морфотипов. Обсуждается вариабельность различных признаков (включая морфологию и экологию) и их таксономическая значимость и применимость на практике. Предлагается набор признаков, который настоятельно рекомендуется использовать в последующих описаниях новых видов *Trichia* и близких родов, а также даются некоторые методические рекомендации по морфологическому анализу.

Ключевые слова: Myxomycetes, Trichiales, видовой комплекс, мультигенная филогения, СЭМ, таксономическая ревизия

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Myxomycetes (Myxomycetes = Myxogastrea) is one of the numerous groups of organisms whose classical systematics was built on the morphology of sporophores, and morphological characteristics were and still are most important for supporting taxonomic revisions and describing new taxa (Nannenga-Bremekamp 1974, Novozhilov et al. 2008, Ronikier et al. 2010, Kuhnt 2021). At the same time, the use of molecular methods in analyzing genetic diversity of myxomycetes and doing phylogenetic reconstructions in combination with morphological characteristics is becoming increasingly common (Leontyev et al. 2015, 2019, Schnittler et al. 2017, Ronikier et al. 2020, 2022). Soil metagenomic studies launched in recent years allowed to isolate thousands of marker gene sequences of myxomycetes from environmental samples (Borg Dahl et al. 2018, Gao et al. 2019, Shchepin et al. 2019, 2021). However, the majority of them still fail to be associated with known morphospecies, mainly for two reasons: 1) insufficient level of molecular studies of already described taxa (for example, only for 8 of 30 described species of genus *Trichia* Haller nrSSU sequences are available in GenBank), and 2) immense hidden genetic diversity even within one morphospecies (Shchepin et al. 2022). All this leads to a high demand for revision of already known groups using a combination of

morphological and genetic methods (a so-called polyphasic approach), in particular, the genus *Trichia*.

The genus *Trichia* was described in 1768, and 30 species are currently recognized within it (Haller 1768, Lado 2005–2023). Sporocarps with one or two closely adhering peridium layers, predominantly yellow spore mass, simple or very scantily branched elaters ornamented with left- (rarely right-) handed spiral thickenings are considered to be diagnostic features of the genus.

Seven species belong to the *Trichia botrytis* complex: *T. ambigua* Schirmer, L.G. Krieglst. & Flatau, *T. botrytis* (J.F. Gmel.) Pers., *T. erecta* Rex, *T. flavicoma* (Lister) Ing, *T. munda* (Lister) Meyl., *T. papillata* Adamonytė, and *T. subfusca* Rex. Similar features of these species are stalked sporangia, two-layered peridium with the outer layer unevenly thickened or divided into plates separated by lighter, usually yellow membranous dehiscence lines, and warted spores. The latest described species of this complex, *T. ambigua*, was distinguished during a morphological revision with special attention to *T. subfusca* (Schirmer et al. 2015).

During our research in the Russian Far East, one intriguing species of *Trichia* resembling a tiny copy of *T. botrytis* was discovered. While studying it, we sought to compare it with the already described species of the complex, using literature data and specimens available at the LE and MYX fungaria. It appeared, however, that many specimens were misidentified and did not correspond to the original descriptions, and simultaneously the same "species" were represented by extremely different morphological specimens. When the obtained sequences of marker genes were compared with the GenBank database, it turned out that there were only 4 nrSSU gene sequences of species from the *T. botrytis* complex: 2 of *T. botrytis* and 1 of *T. erecta* and "*T. subfusca*" (see notes on *T. ambigua*).

Therefore, we realized that we cannot reliably compare a new species with those already described without drawing the boundaries between these species so thoroughly as protologues and available specimens allowed us to. Thus, more than 150 specimens deposited in LE and MYX from Russia and Vietnam, originally assigned to 7 species of the *T. botrytis* complex, were examined during our work. A comprehensive study of their morphological and genetic features yielded 6 of the 7 previously described species of the complex, as well as 10 taxa (9 species and 1 variety) new to science.

We give a detailed description of all the morphotypes of *Trichia* studied, compare them with one another and with other closely related species of the genera *Trichia* and *Hemitrichia* Rostaf. using morphological, ecological, and molecular characteristics, and also discuss variability and taxonomic significance of these characteristics in differentiation of taxa belonging to the family Trichiaceae at species level.

MATERIAL AND METHODS

Specimen collection

Specimens of *Trichia* spp. were obtained during field studies and from moist chamber cultures according to standard protocols (Stephenson 1985, Wrigley de Basanta & Estrada-Torres 2021) and deposited in the LE (St. Petersburg) and MYX (Moscow) fungaria. Specimens from different regions of Russia and Vietnam were collected between 2008 and 2021. A complete list of specimens with herbarium numbers, time and place of collection, collectors, substrates, and their pH (if available) is given in the supplementary materials (S1).

Comparative morphological analysis

The morphological features of fruiting bodies were examined using a Zeiss Axio Zoom V16 stereomicroscope and a Zeiss Axio Imager A1 microscope (using DIC in some cases). For the sake of standardization, semi-permanent slides were prepared in lactophenol under identical conditions and all measurements were performed by the first author.

The ultrastructural features of spores, capillitium, and inner peridium were examined after gold-palladium alloy sputter coating using scanning electron microscopes CamScan S-2 (Cambridge Instruments), JSM-6380LA (JEOL), and Quattro S (Thermo Fisher Scientific) in the Interdepartmental Laboratory of Electron Microscopy at the Faculty of Biology, MSU and JSM-6390LA (JEOL) in the Core Facility Center, BIN RAS. When mentioning high-resolution SEM in the descriptions and notes, we refer to the images obtained with the Quattro S microscope as the most modern and powerful one from the microscopes listed above.

Linear measurements of structures in the (a–) b–c (–d) format always denote Min, Mean–SD, Mean+SD, and Max, respectively, and are indicated in the text without additional explanations.

Terminology used

In all cases, secondary ornamentation of elaters implies ornamentation of the area between spiral bands. If the spiral bands themselves (i.e., elements of the primary ornamentation) have some additional ornamentation, for example in the forms of spines, warts, or fine mesh, it is stated individually and is not included in the concept of "secondary ornamentation of elaters".

Describing the pattern of spore surface ornamentation, we used the terminology introduced by (García-Cunchillos et al. 2021) with one difference: we mentioned heads as components of one caput rather than completely synonymized these terms. Secondary ornamentation of spores refers to the ornamentation of the area between bacula or pila constituting primary ornamentation.

Spore size

To determine the size of spores, slides were photographed using oil immersion and, for additional convenience, a collage with spores of one specimen was created in a graphic editor in one scale. Photographs were taken so that the middle part of the spore with the maximum diameter was in the focal plane. Obviously underdeveloped spores with abnormally large size were ignored. The spore diameter was measured including elements of ornamentation, but caution was taken not to consider the halo resulting from optical distortion. All the measurements of diameter were made strictly in the same direction (in our case, ± vertically) regardless of the actual position of spores on the slide.

Many members of the order Trichiales (and all members of the *Trichia botrytis* complex) have spores that are not perfectly spherical but somewhat irregular, and in some cases

values of both spore length and width are given in the literature. This research technique, however, is twice as labor-intensive, and the results of such measurements are inconvenient to compare with the results of a one-way measurement of the spore diameter, that prevail in the literature and keys to species. In addition, sometimes the difference between the length and the width of the spore is so small that it cannot be surely determined by eye. Therefore, we used a large number of strictly unidirectional measurements that allowed us to account for the entire range of variability in spore diameter in different directions without significant inaccuracy.

Elater width

To measure width of elater, oil-immersion photographs of the elater middle part (never of the tapering ends) were taken so that the widest part of it fell into focus. It is necessary to take elater spiral ornamentation into account, except for clearly prominent spines (as, for example, those of *Trichia erecta* or *Hemitrichia spinifera* M.L. Farr). In some cases, especially if spirals are unevenly thickened or distanced from one another, it is possible to make a mistake by measuring thickness in only one particular place. To avoid this, one should mentally draw two parallel lines through the most protruding parts of the spirals on both sides and measure the distance between these lines. Many programs have the ability to visualize such parallel lines, which simplify the researcher's task. It is necessary to avoid inclusion of obvious optical distortions in the segment as well as in the case of spores. See the example of correct and incorrect measurements in the supplementary materials (Fig. S2).

Elater length

To measure length of elater, photos of the elater using suitable zoom were taken. Objectives with magnification 10× and especially 4× do not often provide a good view of the elater tips and places of its twists and bends, and the one with 100× magnification is usually inconvenient due to the small field of view. Therefore, 20× or 40× lenses are most suitable. If the elater is very short or located in one focal plane on the slide, one photo may be enough to trace all its parts. But more often it is necessary to take several photos in different focal planes and then use a graphic editor to combine fragments that fell into focus. The measurements should be made not just between the ends of elater, but following all its bends with the help of a "broken curve" tool or similar. See the example of correct measurements in the supplementary materials (Fig. S3).

Length of elater tips

To measure the length of elater tips, it is best to take photographs using objectives with magnification of 40× or 100×. One should choose endings that are not too curved and located in the more or less same focal plane, so that their projection corresponds most to their actual length. As in the case of the total elater length, one should use a "broken curve" measuring tool, because tips, especially long ones, are rarely perfectly straight. Base of the tip is the point where the elater thickness begins to decrease and the walls stop being nearly parallel to each other. With the exception of a few species, where tapering is extremely smooth, it is quite easy to see this point, because it is very often marked by a swelling, even if it is not very conspicuous. Frequently, it can be observed that swelling coincides with the ampulliform rounded end of the internal space of the elater. Also, in some cases the elater is characterized by a small bend in the place of the tip base changing direction rather abruptly. It is also worth bearing in mind, that tip bases should not always be looked for directly in the terminal parts of elaters, because, for example, in the case of *Trichia botrytis* s. s., two ends together may make up almost 50 % of the total elater length, and thus the base of each of them is 1/4 length from the tip itself. See example of correct measurements in the supplementary materials (Fig. S4).

Direction of spiral bands

Determination of the spiral band direction requires observing elaters at high magnification in such a way that the surface closest to the researcher's eye falls into focus. Since elaters are translucent, it is easy to see the other side of the elater with spirals going in the opposite direction, which will lead to a mistake. It is also recommended to look not at the ends, but at the middle part of the elater, where thickness and distribution of spirals are most even. See the explanation in Poulain & Meyer (2007) or Poulain et al. (2011) as well as in supplementary materials (Fig. S5).

DNA extraction, sequencing and phylogenetic analysis

Extraction of genomic DNA was performed from matured air-dried fruiting bodies without a trace of fungal contamination. Approximately 2–5 sporocarps were placed in 2 ml test tubes with screw cap with addition of ceramic balls 3 mm in diameter and frozen at -20°C for at least 30 min. Afterwards, samples were crushed in a Bioprep-24 homogenizer (Hangzhou Allsheng Instruments, Hangzhou, China). DNA was extracted with either a PhytoSorb kit (Sintol, Moscow, Russia) according to the manufacturer's protocol with minor modifications (spore homogenate was eluted with 450 µl of extraction buffer; lysis buffer was added without preliminary precipitation step and supernatant transfer into a new sterile tube; final elution volume was 80–100 µl) or a MagPure Plant DNA Kit (Magen Biotechnology, Guangzhou, China) according to the manufacturer's protocol using an automated DNA extraction station Auto-Pure 96 (Hangzhou Allsheng Instruments, Hangzhou, China).

To reconstruct the phylogeny, two unlinked genetic markers were sequenced. A fragment of approximately 550 base pairs from the 5' end of the nuclear 18S rDNA gene (nrSSU) that is free of introns was obtained with forward primers S1 or NUSSUF3 (Fiore-Donno et al. 2008, Feng & Schnittler 2015) and reverse primers SR4Bright (Fiore-Donno et al. 2008) or SSU_rev (Prikhodko et al. 2023). Fragment of the protein-coding gene for the translation elongation factor 1-alpha (EF1α) was amplified with a set of primers for a semi-nested PCR EF03(EF04)/KEFR3 (Wrigley de Basanta et al. 2017, Ronikier et al. 2020, exon fragment ca. 1075 bp). For two specimens of *Trichia ambigua* and one specimen of *T. flavicoma*, primers 1Fcyt and E500R (Feng & Schnittler 2015) were also used, which allowed us to obtain a longer gene fragment from two overlapping sequences (Table 1).

Additionally, mitochondrial 16S rRNA gene (mtSSU) sequences were obtained for all studied *Trichia botrytis* complex species and the type species of the genus (*T. varia*

Table 1. Primer pairs and amplification protocols used in this study.

Name	F/R	Sequence (5'–3')	Amplification protocol
S1	F	AACCTGGTTGATCCTGCC	5 min at 95°C, 30–36 cycles (30 sec at 95°C, 20 sec at 56°C, 50 sec at 72°C) and 5 min at 72°C
NUSSUF3	F	CCTGCCAGAATCATATGCTTGTC	
SR4Bright	R	TGCTGGCACCAGACTTGT	
SSU_rev	R	AGACTTGTCCTCYAATTGTTAC	
EF03	F	TGATCTACAAGTGCGGTG	5 min at 95°C, 35 cycles (30 sec at 95°C, 30 sec at 60°C, 120 sec at 72°C) and 10 min at 72°C
KEF_R3	R	CCGTTCTTGATGTTCTTGG	
EF04	F	TGGGTGTTGGACAAACTC	5 min at 95°C, 30 cycles (30 sec at 95°C, 30 sec at 60°C, 120 sec at 72°C) and 10 min at 72°C
KEF_R3	R	CCGTTCTTGATGTTCTTGG	
Kmit_F	F	AGTGTTATTCGTGATGACTGG	5 min at 95°C, 32 cycles (30 sec at 95°C, 1 min at 52°C, 90 sec at 72°C) and 10 min at 72°C
Kmit_R	R	CGAATTAAACCACATCTCCACC	

(Pers. ex J.F. Gmel.) Pers.) with a Kmit_F/Kmit_R primer pair (Lado et al. 2022, 400–560 bp). As in the previously published paper (García-Cunchillos et al. 2022), we were unable to successfully amplify the gene fragment using the Kmit_Fi nested forward primer.

PCR reactions were prepared with 10 µl of 2 × Bio-Master HS-Taq PCR-Color reaction mix (Biolabmix, Novosibirsk, Russia) containing 100 mM KCl, 0.4 mM dNTPs, 4 mM $MgCl_2$, 0.06 U/µl TaqDNA polymerase, 0.2 % Tween20, and several dyes (xylene cyanol, bromphenol blue, OrangeG, and tartrazine) with addition of 3 nmol of each primer, 1–3 µl of template DNA, and diH_2O up to a total volume of 20 µl. The amplification was carried out with a C1000 Touch (Bio-Rad, Hercules, USA) thermal cycler. Products of amplification were stained with SYBR Green I (Lumiprobe Rus, Moscow, Russia), separated by 1.2 % agarose gel electrophoresis, and observed in Gel Doc XR+ System (Bio-Rad, Hercules, USA), then purified using a CleanMag DNA (Evrogen, Moscow, Russia) purification kit before semi-nested PCR or sequencing with a BrilliantDye Terminator v3.1 Cycle Sequencing Kit (NimaGen, Nijmegen, the Netherlands) using the primers mentioned earlier. Sequencing products were purified with a NimaGen D-Pure DyeTerminator Cleanup kit and analyzed on ABI 3500 automated DNA sequencer (Applied Biosystems, Foster City, USA) equipped with a standard 50 cm capillary array.

Alignments, model selection and phylogenetic analyses

NrSSU and EF1α sequences were compiled into two single-gene alignments in Unipro UGENE (Okonechnikov et al. 2012) and aligned using MAFFT online service (Katoh & Standley 2013, Katoh et al. 2019) with E-INS-I option for nrSSU and G-INS-i for EF1α with default gap penalties. Exon parts of EF1α sequences were determined according to the known nucleotide and protein EF1α sequences of *Tubifera ferruginosa* (Batsch) J.F. Gmel. (GenBank EF513201, Fiore-Donno et al. 2010) and *Trichia varia* (GenBank ON693898, García-Cunchillos et al. 2022).

After trimming of the primer sequences and manual editing two sets of nucleotide sequences were merged into a single alignment with two partitions using SequenceMatrix 1.9 (Vaidya et al. 2011). In the partition file, the section corresponding to the EF1α fragment included only the protein-coding fragments of the gene. Maximum likelihood (ML) analyses were performed using IQ-TREE 1.6.12 (the last stable release, Nguyen et al. 2015) launched on the local machine. The GTR+F+I+G4 evolutionary model was selected for nrSSU partition and TIM2e+I+G4 for EF1α partition according to the ModelFinder tool implemented in IQ-TREE (Kalyaanamoorthy et al. 2017). One thousand ultrafast bootstrap (UBS) replicates (Hoang et al. 2018) were performed to obtain confidence values for the branches. Bayesian inference (BI) was performed with the same dataset using MrBayes 3.2.7a (Huelsenbeck & Ronquist 2001) run on CIPRES Science Gateway (Miller et al. 2010). The phylogenetic analysis was run four times as four separate chains for 10×10^6 generations (sampling every 1000). The convergence of MCMC chains was estimated using Tracer 1.7.2 (Rambaut et al. 2018) and by the average standard deviation of split frequencies; based on the estimates, the first 25 % generations were discarded as burn-in. Posterior probabilities (PP) for clades were exported to the ML-tree. Phylogenetic tree with combined supports was visualized using FigTree 1.4.4 and edited using CorelDRAW 24.0.

RESULTS

A comprehensive study of 154 specimens from the LE and MYX fungaria resulted in the discovery and analysis of 16 distinct *Trichia* morphotypes. For a complete list of studied specimens, including GenBank accession numbers, and a map with their collection localities, see supplementary materials (S1, S6).

Phylogenetic analysis

By the time this study started, only 4 sequences of the *Trichia botrytis* complex species were available in GenBank. The most recent paper with a phylogeny of Trichiales (García-Cunchillos et al. 2022) did not include any species of the complex, so their phylogenetic position remained unknown. Thus, for this publication we obtained 77 nrSSU sequences, 71 EF1α sequences, and 21 mtSSU sequences (three alignments with sequences see S12).

The final two-gene alignment included sequences from 131 specimens belonging to species of the order Trichiales and from two specimens of *Tubifera* J.F. Gmel. species (Reticulariales) comprising the outgroup. For several species, such as *Hemitrichia clavata* (Pers.) Rostaf. (a type species of the genus *Hemitrichia*), *Trichia alpina* (R.E. Fr.) Meyl., and *T. gradalia* **sp. nov.** (see further), all primer combinations

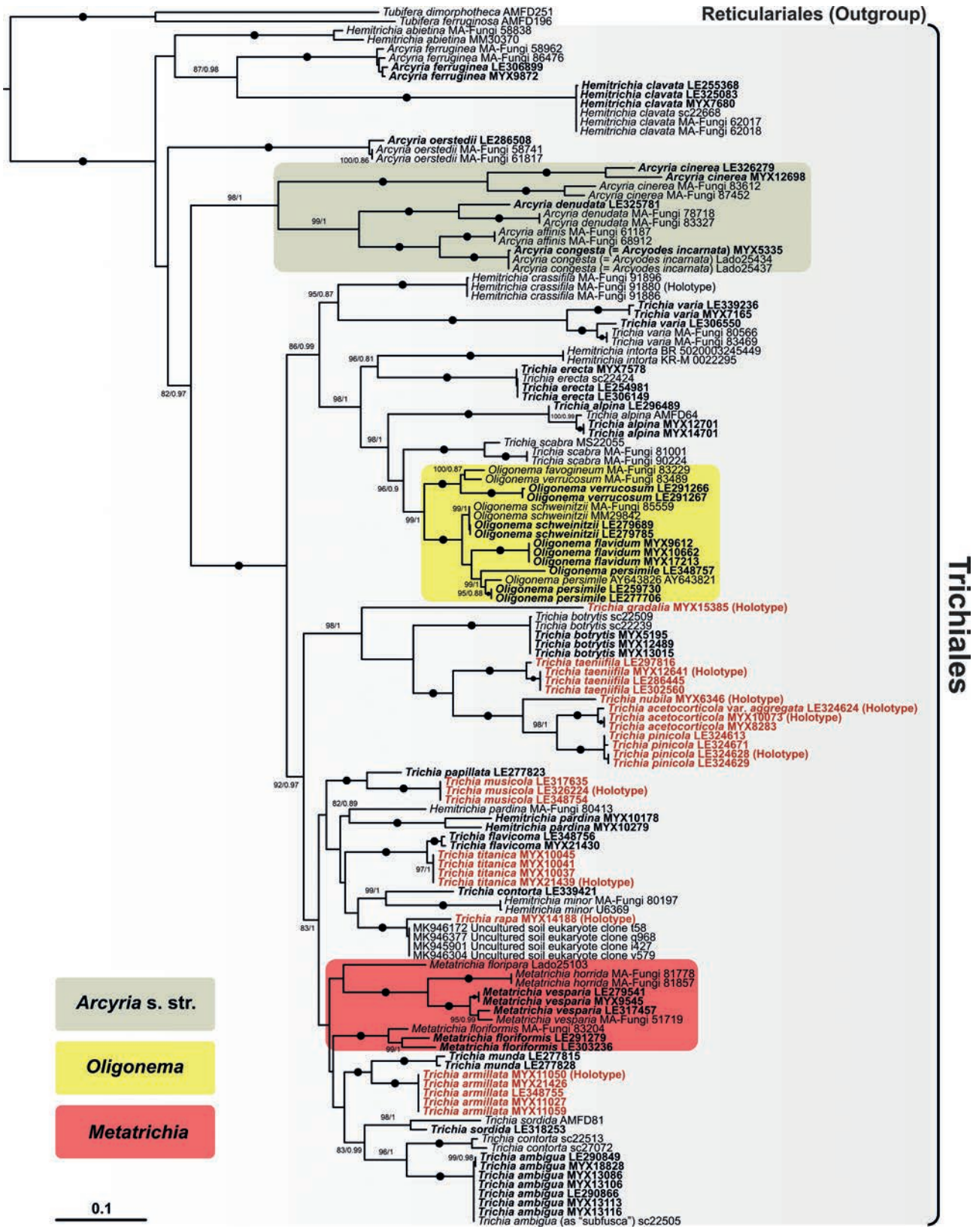

Figure 1 Phylogenetic tree for species of the order Trichiales, obtained from combined nrSSU and EF1α sequences. Branch supports are shown only for UBS/PP ≥ 80/0.8; black dots indicate maximum supports in both analyses (UBS/PP = 100/1). Scale bar represent the mean number of nucleotide substitutions per site. The sequences obtained anew are shown in bold. The type specimens of 9 new species for which nucleotide sequences were obtained are indicated in red bold type

mentioned did not allow efficient amplification or sequencing of EF1α gene fragment, so the concatenated alignment for ML and BI analyses included 133 nrSSU sequences and 112 EF1α sequences with 95 and 1064 (without considering the intron sequences) sites, respectively. The alignment fragment directly involved in the phylogeny reconstruction included

1999 columns with 1097 distinct patterns, 151 singleton sites, and 1037 constant (non-informative) sites in total. The topologies of ML and BI phylogenies were congruent, and the clades that received maximum statistical support with UBS and PP were mostly identical (Fig. 1).

Species of the genera *Oligonema* Rostaf., *Metatrichia* Ing, *Trichia* p.p., and *Hemitrichia* excl. *Hemitrichia* s. s. form a distinct monophyletic clade (UBS/PP = 100/1.00). They all differ from the more basal *Hemitrichia* s. s. and *Arcyria* F.H. Wigg. s. s. in that their stalk, if present, contains amorphous matter, but never spore-like cysts.

The aforementioned monophyletic clade divides into two large subclades. The first one (UBS/PP = 86/0.99) includes only one of the 16 *Trichia* morphotypes studied in this article, *T. erecta*, representatives of the monophyletic (UBS/PP = 99/1.00) genus *Oligonema*, two representatives of *Hemitrichia* excl. *Hemitrichia* s. s., *H. crassifila* and *H. intorta*, and three other species of the genus *Trichia*: *T. alpina*, *T. scabra* Rostaf., and *T. varia* (a type species of the genus). At least two large clusters can be distinguished in this subclade.

The second subclade (UBS/PP = 92/0.97) includes 15 of the 16 *Trichia* morphotypes studied, representatives of the paraphyletic genus *Metatrichia*, two representatives of *Hemitrichia* excl. *Hemitrichia* s. s., *H. minor* G. Lister and *H. pardina* (Minakata) Ing, and two other *Trichia* species, *T. contorta* (Ditmar) Rostaf. and *T. sordida* Johannesen. The topology of the major branches within this clade is still not fully resolved, except for a well-formed group of *T. botrytis* s. s. and five new species.

Species descriptions

Morphological descriptions of the studied species are given in such an order so that they correspond to their position on phylogenetic tree for easier and faster comparison of related species. The Habitat and Distribution sections include only specimens studied by us and specimens, whose nrSSU sequences presented in GenBank fully agreed with ours, since not all the literature references can be unambiguously attributed to the morphotypes discussed herein.

Trichia erecta Rex Figs 2, S6–S11

Description. Sporocarps stalked sporangia, solitary or in scattered groups, 760–2200 µm high. Stalk slightly tuberculous or finely furrowed, comparatively long, 380–1480 µm high, 42–68 % TH, light brown to dark brown, evenly colored, filled with refuse matter. Sporotheca globose or subglobose (slightly elongated in the lower part), 330–700 µm wide. Peridium two-layered. Inner layer thin, membranous, hyaline; decorated with shallow oblique lines, spaces between lines look furrowed in transmitted light (TL). Under SEM, scale-like to slate-like pattern can be seen. Outer layer made of granular matter, light brown to reddish-dark-brown; peridium plates abundant, usually no less than 10 on a visible part of sporotheca. Dehiscence by preformed lines. Capillitium consisting of bright yellow elaters, pale yellow in TL, always simple, often intertwisted in double spirals, (190–)330–550 (–700) µm (n = 112) long and (3.6–) 4.0–4.7 (–5.1) µm (n = 85) wide. Tips of elaters very short, ca. 7(4–8) µm long, with abrupt conical ends, rarely with short points. Capillitial threads decorated with left-handed spiral bands, quite evenly arranged, with ca. 9–11 turns per 20 µm. By LM, spirals wide, sometimes longitudinally split in two. Oil immersion reveals secondary ornamentation of longitudinal and slightly oblique striae between spirals. Spiral bands usually decorated with short spines, but rarely lack any ornamentation. Under SEM, spines usually short, but can sometimes reach 2 µm long; spiral bands thinner near the base and wider at the periphery. Secondary ornamentation of thin furrowed ridges, going somewhat obliquely to the elater longitudinal axis. Spores bright yellow in mass, pale yellow in TL, usually subglobose, but sometimes of somewhat irregular shape, unevenly warted, large, (11.3–) 12.1–13.4 (–14.6) µm diam. (Mean = 12.78; SD = 0.64; n = 416). Under SEM, spore ornamentation baculate: ends of bacula bumpy, unevenly swollen; spaces between bacula either smooth or with tuberculous secondary ornamentation. Plasmodium not observed.

Habitat: rotten wood, mainly coniferous (in the field).

Distribution: Russia: European part (Murmansk and Tver regions), West Siberia (Republic of Altai, Altai Territory); Germany (Bavaria).

Studied specimens: see table S1.

Notes: the specimens studied match the original description of *Trichia erecta* (Rex 1890). The only minor difference is the elaters slightly wider than those described by Rex: (3.6–) 4.0–4.7 vs 3.75–4.0 µm.

Trichia erecta is a very distinctive, easy-to-identify species, which seems to be uniformly understood by many authors unlike other species of the *T. botrytis* complex. Identification is facilitated by the specific features, such as large spores up to 13–14 µm diam. (the largest ones among the specimens of *Trichia* we studied) and characteristic elaters with spiral bands decorated with fine spines and very short tips no longer than 8 µm. However, these spines may be occasionally absent (e.g., Martin & Alexopoulos 1969). One of the studied specimens, LE 306149, had smooth spiral bands (Figs 2f, j), but in every other feature, including the nrSSU sequence, it was identical to others we attributed to *T. erecta*. However, it was the only specimen for which we failed to sequence the EF1α gene fragments several times.

Despite the external resemblance to other species of the *Trichia botrytis* complex, *T. erecta*, surprisingly enough, is closely related to species of the *Trichia favoginea* complex, which have been recently transferred to the genus *Oligonema* (García-Cunchillos et al. 2022), as well as to *Hemitrichia crassifila* A. Ronikier & Lado and *H. intorta* (Lister) Lister, which is shown by phylogenetic analysis (Fig. 1). Although habits of these species differ a lot, there are micromorphological features that bring them together: ornamentation of the inner peridium and capillitium. The inner peridium is truly distinctive and unlike all other species of the *T. botrytis* complex. By LM and especially under SEM, it resembles fine reptile scales: compare *T. erecta* (Figs 2n, o, Rammeloo 1974a, fig 40), *Oligonema affine* (de Bary) García-Cunch., J.CA. Zamora & Lado, *O. favogineum* (Batsch) García-Cunch. Zamora & Lado, *O. persimile* (P.Karst.) García-Cunch., J.CA. Zamora & Lado (Rammeloo 1974a, fig. 23, 41, 45), *Hemitrichia crassifila*, and *H. intorta* (Ronikier et al. 2020, fig. 7 B, G). Capillitium of these species is typically decorated with fibrous striae between spiral bands, which can be observed by both LM and especially SEM: compare *T. erecta* (Figs 2i–k, Neubert et al. 1993, p. 299, fig. d), *O. affine* (Camino et al. 2007, fig. 2, 3), *O. persimile*, *T. scabra* (Neubert et al. 1993, p. 301, fig. a, d), *H. serpula* (Scop.) Rostaf. ex Lister (Neubert et al. 1993, p. 293, fig. f, García-Cunchillos et al. 2021), *H. crassifila*, and *H. intorta* (Neubert et al. 1993, p. 292, fig. e, Ronikier et al. 2020, fig. 7 D, E, I, J).

Trichia gradalia Bortnikov & Gmoshinskiy **sp. nov.**
Figs 3, S6–S11

MycoBank: 848470.

Etymology: from Medieval Latin *gradalis* – "cup, goblet". Refers to the shape of sporangia: subglobose sporotheca and stalk resemble a precious goblet.

Description. Sporocarps stalked sporangia, scattered or in sparse groups, 1050–1770 µm high. Stalk deeply furrowed, 510–920 µm high, 47–52 % TH, light brown to dark brown, occasionally unevenly colored (lighter near the base, darker at the top), filled with refuse matter; furrows of the stalk often spread to the sporotheca. Sporotheca usually single, obovate to globose, 490–690 µm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, tuberculous or punctate due

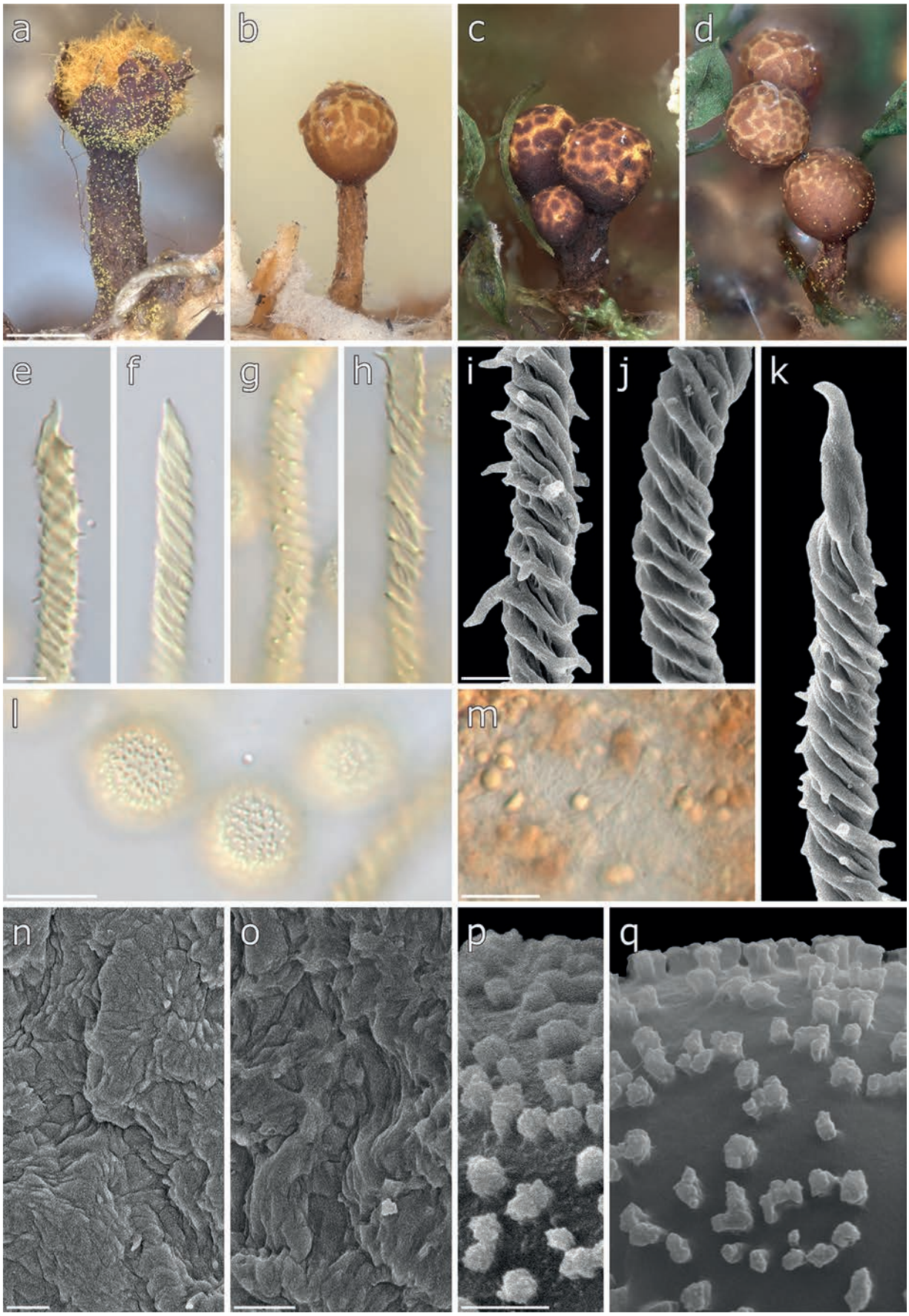

Figure 2 *Trichia erecta* Rex (a–q): a–d – sporocarps, e, f – tips of elaters (LM), g, h – elaters (LM), i, j, k – elaters (SEM), l – spores (LM), m – peridium (LM), n, o – inner peridium (SEM), p, q – spore ornamentation (SEM). a, m – from MYX 4963, b, e, g, l, n, q – from LE 254981, c, f, j – from LE 306149, d, h, i, k, o, p – from MYX 7578. Scale bars: a–d – 500 μm, l, m – 10 μm, e–h – 5 μm, i–k, n, o – 2 μm, p, q – 1 μm

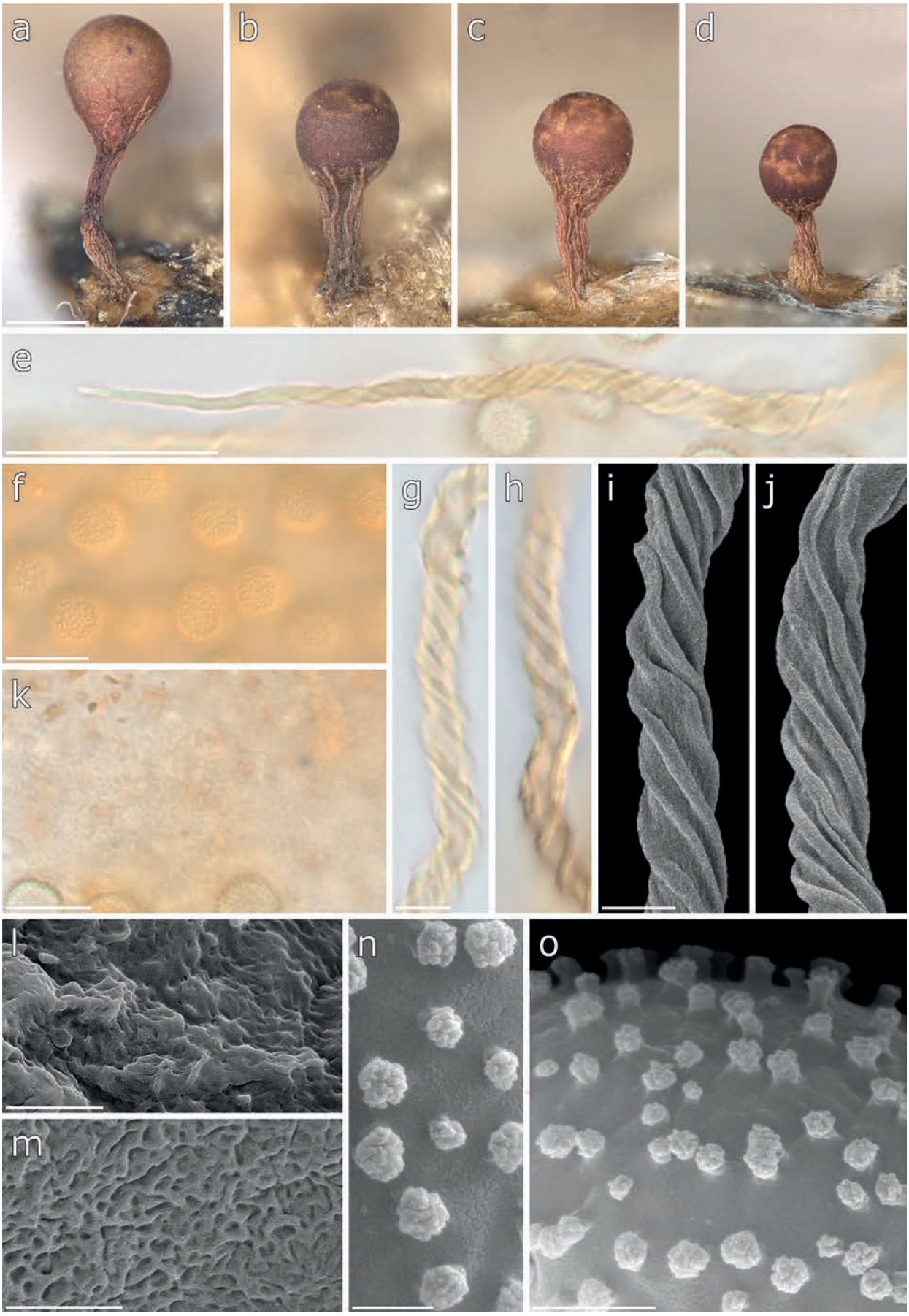

Figure 3 *Trichia gradalia* **sp. nov.** (a–o): a–d – sporocarps, e – elater tip (LM), f – spores (LM), g, h – elaters (LM), i, j – elaters (SEM), k – peridium (LM), l, m – inner peridium (SEM), n, o – spore ornamentation (SEM). a–o – from MYX 15385 (holotype). Scale bars: a–d – 500 μm, e – 20 μm, f, k – 10 μm, g, h, l, m – 5 μm, i, j – 3 μm, o – 1 μm, n – 0.5 μm

to ornamentation of dots or rods by LM. Under SEM, ornamentation strongly tuberous, with ellipsoid invaginations of various shape. Outer layer made of granular matter, light brown to reddish-dark-brown, dehiscence lines faded due to the gradual thinning of deposits, sometimes peridium plates not pronounced. Dehiscence by preformed lines or almost irregular when peridium plates not pronounced. Capillitium consisting of reddish-yellow elaters, pale yellow in TL, always simple, (130–) 350–660 (–870) µm (n = 28) long and (4.0–) 4.1–4.8 (–5.0) µm (n = 15) wide. Tips of elaters medium, ca. 44 (25–58) µm long, gradually tapering to a pointed end. Capillitial threads decorated with left-handed, thin spiral bands, comparatively sparsely and evenly arranged, ca. 7–9 turns per 20 µm. Spiral bands smooth, secondary ornamentation almost indistinct or represented by little roughness or hardly seen longitudinal striae, poorly visible by both LM and SEM. Spores light yellow in mass, pale yellow in TL, usually subglobose, but often of somewhat irregular shape, warted, (7.7–) 8.3–9.0 (–9.2) µm (Mean = 8.6; SD = 0.34; n = 84). Under SEM, spore ornamentation pilate: pila distanced from one another, very seldom drawn together. Secondary ornamentation of tiny verrucae arranged in both sparse groups with distinct individual elements and gregarious groups with partly coalescing verrucae. Plasmodium not observed.

Habitat: rotten wood (in the field).

Distribution: Russia: European part (Republic of Karelia).

Holotype: RUSSIA, Republic of Karelia, vicinity of White Sea Biological Station, young birch-wood with spruce and pine, 66.547923°N 33.1307020°E, 26.08.2017, leg. V.I. Gmoshinskiy, N.I. Kireeva, on dead deciduous bark (MYX 15385).

Notes: this species bears a strong resemblance to *Trichia botrytis*, and therefore was originally identified as such. However, these species have a number of clearly different features.

First, the elater tips of *Trichia gradalia*, although also very gradually tapering, are much shorter compared to *T. botrytis* (44 µm vs 84 µm avg.), and even the ranges of these measurements do not overlap (25–58 µm vs 69–102 µm). Second, spiral bands of *T. gradalia* elaters are narrower and have no fibrous striae. Third, ornamentation of the inner peridium is different: it is composed of invaginations in the form of dots or slightly elongated rods in the case of *T. gradalia*, and in the form of longer rods or sometimes of a fine mosaic pattern of connecting lines in the case of *T. botrytis*. Fourth, both spore ornamentation and size differ greatly. Spores of *T. gradalia* bear separated minute pila and tiny secondary ornamentation, visible only under high-definition SEM. The ranges of *T. gradalia* and *T. botrytis* spore diameter overlap only in extreme values, while the average size of the former diameter is noticeably smaller: (7.7–) 8.3–9.0 (–9.2) µm compared to (8.6–) 9.5–10.6 (–11.9) µm. Fifth, the sporotheca base of *T. gradalia* is often blunter than that of *T. botrytis*, with deep furrows extending from the stalk.

The spore diameter, comparatively small within the genus *Trichia*, is one of the diagnostic features of *T. gradalia*. Another species with small spores is *Trichia microspora* Yu Li & Q. Wan described from China (Li et al. 1989). However, it has spores even slightly smaller than *T. gradalia* has: 7.5–8.2 (–9.1) µm vs (7.7–) 8.3–9.0 (–9.2) µm. This species also differs in other traits: dark brown rather than light yellow spores in mass, long stalk of 1110–1300 µm (510–920 mm in *T. gradalia*), and elaters with more pronounced secondary ornamentation of longitudinal striae.

Trichia botrytis (J.F. Gmel.) Pers. Figs 4, S6--S11

Description. Sporocarps stalked sporangia, scattered, sometimes in large groups, 1230–1730 µm high. Stalk deeply furrowed, 390–820 µm, 29–52 % TH, sometimes gradually colored: brown near the base and almost black near the sporotheca, filled with refuse matter. Sporotheca obovate, 580–830 µm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with abundant short striae, intersecting at different angles and sometimes intersecting, thus forming a mosaic pattern. Outer layer made of granular matter, dark brown to reddish-dark-brown and almost black; dehiscence lines faded due to the gradual thinning of deposits. Dehiscence by preformed lines. Capillitium consisting of yellow or rusty yellow elaters, pale yellow in TL, always simple, (240–) 295–380 (–485) µm (n = 121) long and (3.7–) 4.1–5.1 (–6.5) µm (n = 61) wide. Tips of elaters extremely long, ca. 84 (69–102) µm long, very gradually tapering to a pointed end. Capillitial threads decorated with evenly arranged, quite thick, left-handed spiral bands, ca. 7–9 turns per 20–µm. By LM, spirals evenly and densely packed. Under SEM, spiral bands very thick and densely packed, so that the elater surface almost invisible; spirals often longitudinally split, fibrous. Secondary ornamentation absent. Spores yellow in mass, pale yellow in TL, usually subglobose, but sometimes of somewhat irregular shape, finely warted, (8.6–) 9.5–10.6 (–11.9) µm diam. (Mean = 10.08; SD = 0.55; n = 208). Under SEM, spore ornamentation pilate: pila very seldom solitary, usually arranged in clusters or short lines, joined by thin bridges between caputs or by lateral sides of nearby caputs. Secondary ornamentation absent. Plasmodium not observed.

Habitat: rotten wood (in the field).

Distribution: Russia: European part (Republic of Karelia, Pskov and Tver regions), Germany (Bavaria, Saxony).

Studied specimens: see table S1.

Notes: *Trichia botrytis* is a very widely distributed species described, according to Hagelstein (1944), from Germany in the 13th edition of Systema naturae (Gmelin 1792). The original description of both basionym (*Stemonitis botrytis* J.F. Gmel.) and combinations published in 1792 and 1794, respectively (Gmelin 1792, Persoon 1794), are extremely brief and contain no precise data on the size of certain structures, so we take by the conception of this species generally accepted in several monographs as a reference.

Thus, Lister (1894) and Meylan (1927) indicate that "typical" *Trichia botrytis* is characterized by large sporangia, elaters with very long, gradually tapering ends (50–70 µm and 75–100 µm long, respectively), and spores 9–11 and 9–12 µm diam., respectively. The elater length is not given in the vast majority of publications, except for one: Vasyagina et al. (1977) states that capillitial threads are 250–350 µm long.

Previously published data fairly agree with our measurements: elater tips are 84 µm long avg. (from 69 to 102 µm), total elater length averages 295–380 µm, spore diameter is (8.6-) 9.5–10.6 (–11.9) µm, and elater width is 4.1–5.1 µm avg. (Lister 1894, Martin & Alexopoulos 1969, Ing 1999). It is worth adding that the only nrSSU sequences of *Trichia botrytis* available in GenBank (KT358718 and KT358719 obtained from specimens from Germany) show zero and one mismatches, respectively, and cluster with ours with maximum support. Therefore, we confidently attribute our specimens to *T. botrytis* s. s.

There is also a "mysterious" species – *Hemitrichia botrytis* Georgev. – which is cited by Lado (Lado 2001, 2005–2023) on the basis of a brief mention in Petrak's list and is completely unknown to scientific community. However, this species, described by Petar Đorđević from Mt. Goč in Serbia, is actually similar to *Trichia botrytis* only by its name. The original description of this species states that its stalks contain spore-like cysts, which distinguishes *H. botryits* from all species of the *T. botrytis* complex and definitely places it in the genus *Hemitrichia* s. s. (Đorđević 1929). It is also worth mentioning that, due to the lack of a type material, this name has been proposed to be treated as *nomen nudum* (Ing & Ivancevic 2000).

Trichia taeniifila Bortnikov **sp. nov.** Figs 5, 6, S6–S11

MycoBank: 848471.

Etymology: from *taenia* – "ribbon"; extremely sparse and long spiral bands do not function as stiffeners, so elaters often become soft and flat like ribbons.

Description. Sporocarps stalked sporangia, scattered, 890–1490 µm high. Stalk tuberculous, 340–660 µm high, 36–56 % TH, light brown to almost black, sometimes reddish, sometimes slightly shiny, filled with refuse matter. Sporotheca singe or by two on a common stalk, subglobose to obovate, 470–600 µm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, punctate due to ornamentation of invaginated points and rods by LM. Under SEM, ornamentation seems to be composed of short lines not for-

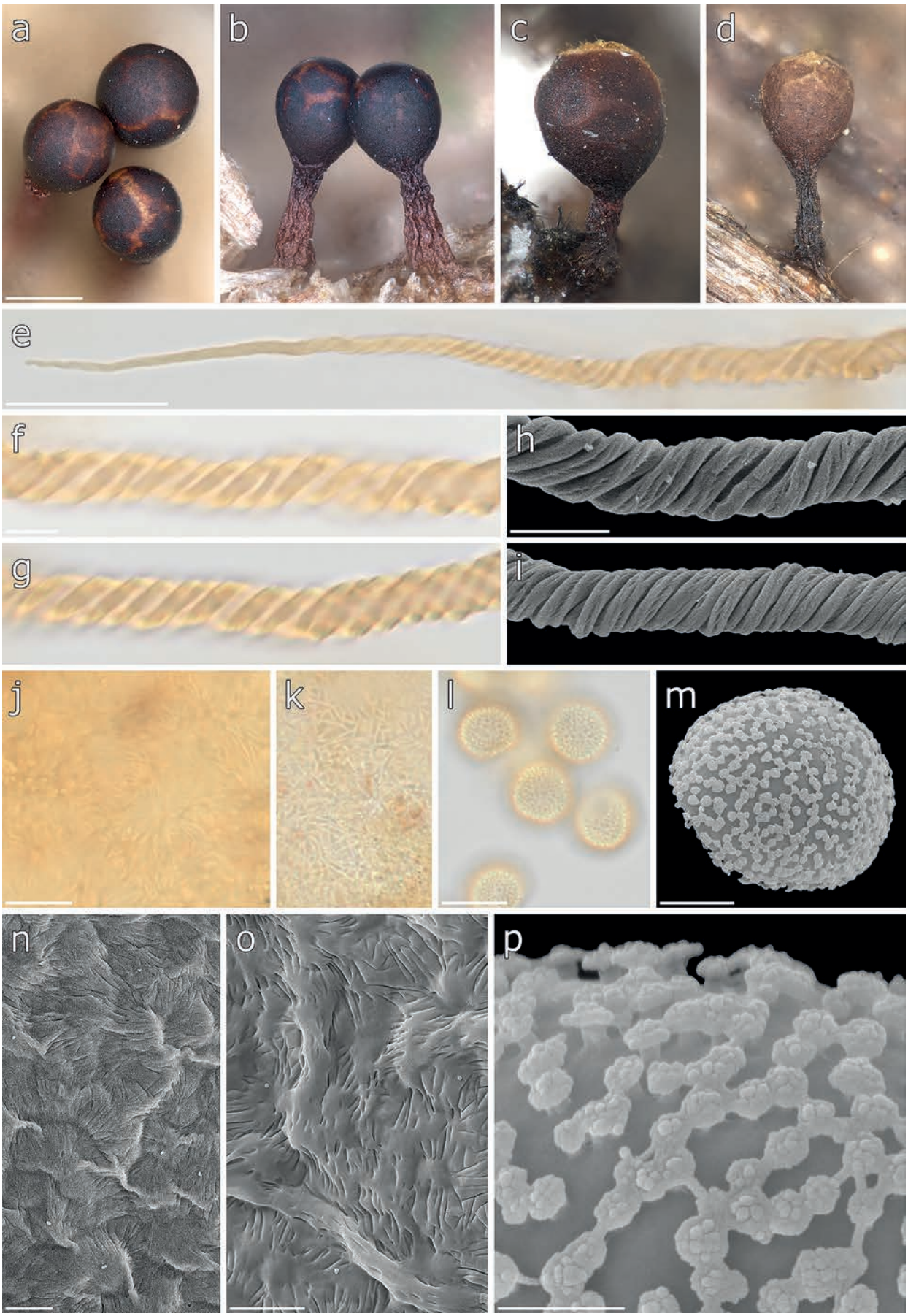

Figure 4 *Trichia botrytis* (J.F. Gmel.) Pers. (a–p): a–d – sporocarps, e – elater tip (LM), f, g – elaters (LM), h, i – elaters (SEM), j, k – peridium (LM), l – spores (LM), m – spore (SEM), n, o – inner peridium (SEM), p – spore ornamentation (SEM). a, b, e, f, g, i, j, k, l, m, o, p – from MYX 12489, c – from MYX 5195, d, h, n – from MYX 13015. Scale bars: a–d – 500 μm, e – 20 μm, j, k, l – 10 μm, f, g, h, i, n, o – 5 μm, m – 3 μm, p – 1 μm

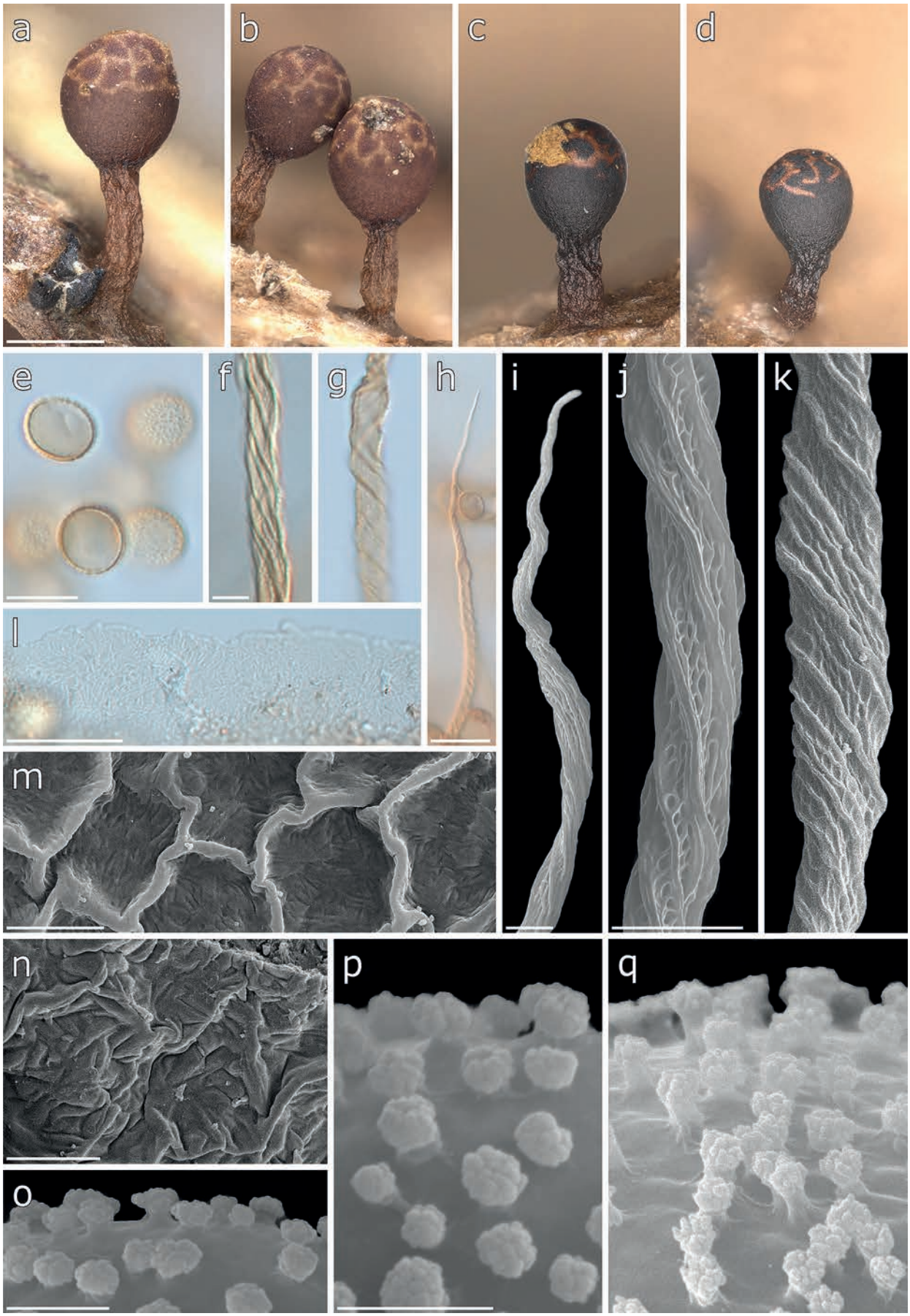

Figure 5 *Trichia taeniifila* **sp. nov.** with left-handed spiral bands (a–q): a–d – sporocarps, e – spores (LM), f, g – elaters (LM), h, i – tips of elaters (LM and SEM), j, k – elaters (SEM), l – peridium (LM), m, n – inner peridium (SEM), o, p, q – spore ornamentation (SEM). a, b, e, g, h, k, n, q – from LE 297816, c, d, f, I, j, l, m, o, p – from MYX 12641 (holotype). Scale bars: a, b, c, d – 500 μm, h, l – 20 μm, e – 10 μm, f, g, I, j, k, m, n – 5 μm, o, p, q – 1 μm

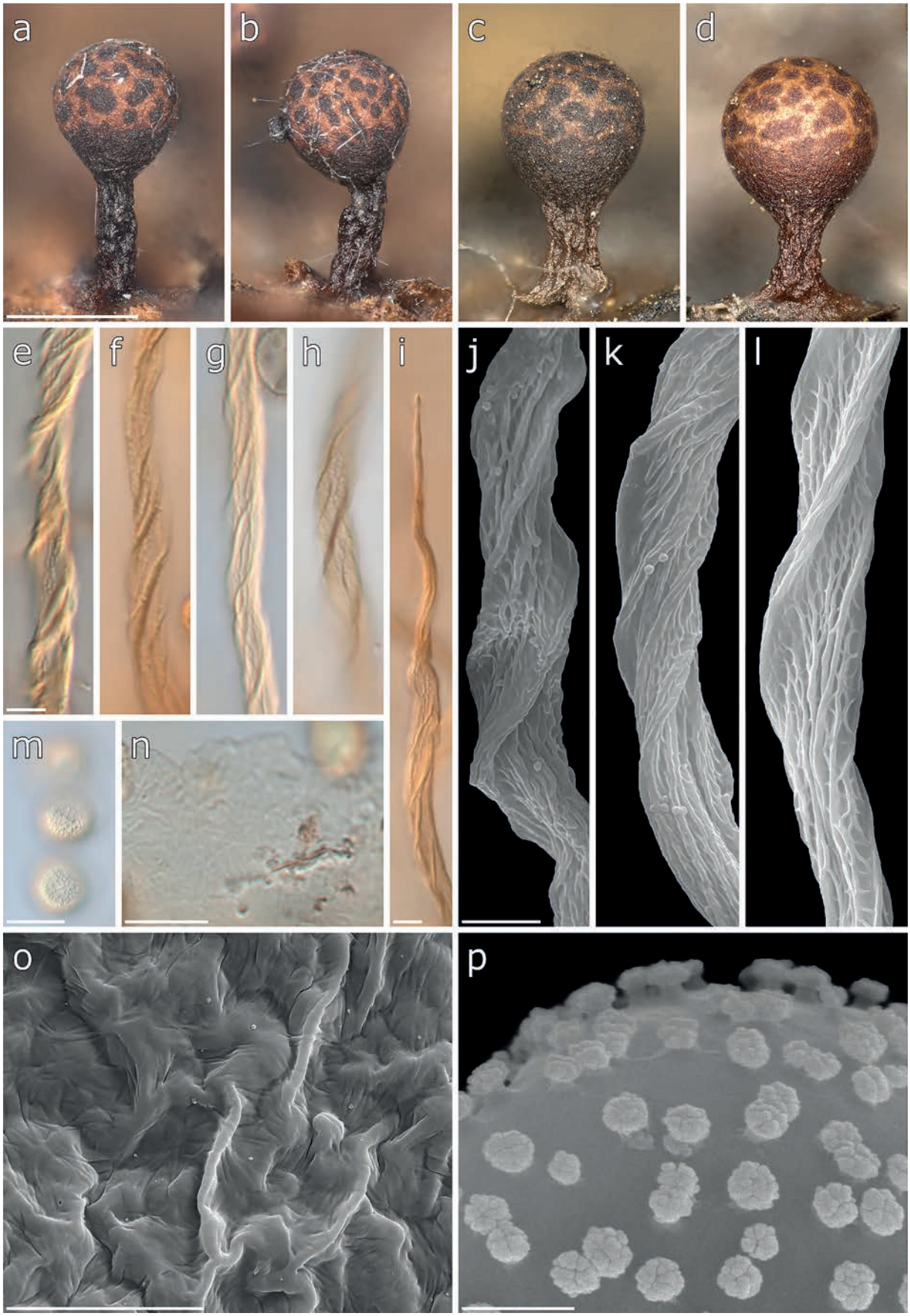

Figure 6 *Trichia taeniifila* **sp. nov.** with right-handed spiral bands (a–p): a–d – sporocarps, e, f, g, h, i – elaters (LM), j, k, l – elaters (SEM), m – spores (LM), n – peridium (LM), o – inner peridium (SEM), p – spore ornamentation (SEM). a, b, j – from MYX 11405, c, d, g, h, I, l, m, n, o, p – from LE 286445, e, f, k – from MYX 21432. Scale bars: a–d – 500 μm, m, n, o – 10 μm, e, f, g, h, i – 5 μm, j, k, l – 3 μm, p – 1 μm

ming a complete net. Outer layer made of amorphous material, brown to reddish-dark-brown or almost black; peridium plates usually quite contrasting and abundant, with more or less distinct margins. Dehiscence by preformed lines. Capillitium consisting of dark yellow to rusty-yellow elaters, pale yellow in TL, always simple (240–) 360–630 (–950) μm (n = 123) long and (3.9–) 4.5–5.7 (–6.5) μm wide (n = 78). Tips of elaters medium, ca. 51 (32–67) μm long, gradually tapering to a pointed end. Capillitial threads decorated with thin left-handed or right-handed spiral bands (always in one direction within one specimen), unevenly arranged, quite distanced from one another, directed along the elater longitudinal axis, ca. 2–6 (–7) turns per 20 μm. Secondary ornamentation of thin spirals and a reticulum by LM. Under SEM, spiral bands represented by short thin ridges, oblique and sometimes furrowed, with a small-meshed net between spirals, sometimes as high as the elements of primary ornamentation. Spores yellow to bright yellow in mass, pale yellow in TL, usually subglobose, but sometimes of somewhat irregular shape, warted, (8.3–) 9.1–10.1 (–11.8) μm diam. (Mean = 9.57; SD = 0.49; n = 327). Under SEM, spore ornamentation pilate: pila distanced from one another, rarely drawn together, but never forming any bridges between caputs. Secondary ornamentation absent. Plasmodium not observed.

Habitat: rotten wood (in the field).

Distribution: Russia: Far East (Primorye Territory).

Holotype: RUSSIA, Primorye Territory, Kedrovaya Pad Nature Reserve, deciduous forest with *Tilia* spp. and *Fraxinus* spp., 43.158111°N 131.467361°E, 16.08.2017, leg. F.M. Bortnikov, E.A. Antonov, on deciduous wood (MYX 12641).

Other studied specimens (paratypes): see table S1.

Notes: The central species of the complex, *Trichia botrytis*, has been previously described in many publications as highly variable, and, apparently, for this reason our specimens of *T. taeniifila* were initially identified as such. However, in fact, *T. taeniifila* has several significant differences from *T. botrytis*.

The most notable difference is the ornamentation of capillitial threads. Elaters of *Trichia taeniifila* are decorated with sparse, elongated spirals, the space between which has a reticulate pattern, clearly visible under both SEM and LM with oil immersion. Since spiral bands are rather relaxed, elaters are often flattened (especially at the bending sites) and have a somewhat ribbon-like shape.

Besides, elaters of *Trichia taeniifila* are longer: 360–630 μm avg. compared to 295–380 μm in case of *T. botrytis*, and, on the contrary, elater tips are shorter: about 51 μm *vs* 84 μm avg. Finally, the pila ornamenting spores of *T. taeniifila* are always separate and not connected by any bridges, as those of *T. botrytis* s. s. This feature, however, is rather difficult to observe without SEM.

A somewhat similar elater ornamentation is found in *Trichia microspora* described from China (Li et al. 1989). This species, however, can be easily distinguished from *T. taeniifila* in other aspects. First, *T. microspora* has a long stalk 1.1–1.3 mm vs 0.4–0.7 mm, comprising more than 50 % TH. Second, *T. microspora* has smaller spores, averaging 7.5–8.2 μm vs 9.1–10.1. Third, the spore mass of *T. microspora* is dark brown rather than yellow as of *T. taeniifila*.

Some studied specimens of *Trichia taeniifila* had unique feature for *Trichia botrytis* complex – right- (not left-) handed spiral bands (Fig. 6). However, in other traits and ecology, specimens with left- and right-handed spiral bands are virtually indistinguishable: collected in the same geographic region, have similar habits and micromorphological characteristics (length and width of elaters, length of elater tips, spore diameter). The obtained nrSSU and mtSSU sequences were also found to be the same, although the EF1α sequence of LE 297816 (left-handed spirals) featured a large number of controversial positions and substitutions. Thus, despite such unique feature, we attribute these specimens to the same species.

Trichia nubila Bortnikov **sp. nov.** Figs 7, S6–S11

MycoBank: 848472.

Etymology: from the Latin nubes – "cloud"; the species is named so because the only known specimen so far was found in Primorye Territory on the slope of Mount Oblachnaya ("cloudy" in Russian). In addition, plates of the outer peridium are rather thin, with blurry, as if cloudy, margins.

Description. Sporocarps stalked or almost sessile sporangia, scattered, 770–1100 μm high. Stalk tuberculous, somewhat furrowed, rather short, 12–34 % TH, reddish-brown, evenly colored, filled with refuse matter. Sporotheca single or by two on a common stalk, obovate, 550–660 μm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with small straight striae, intersecting at different angles and forming a mosaic pattern. Outer layer made of granular matter, brown, dehiscence lines blurry due to the gradual thinning of deposits. Dehiscence by preformed lines. Capillitium consisting of yellow or rusty yellow elaters, pale yellow in TL, simple, extremely long: presumably longer than 1 mm (all elaters on a slide were ripped; observed fragments, ripped from one or two sides, were 400–1800 μm long (795 μm avg.)). Elaters quite wide, (5.8–) 6.1–6.8 (–7.0) μm (n = 18). Tips of elaters short, ca. 16 (13–18) μm long, tapering to a pointed end. Capillitial threads decorated with left-handed spiral bands, quite unevenly arranged, with ca. 6–8 (–9) turns per 20 μm. By LM, spirals somewhat sharped and occasionally longitudinally split. Oil immersion reveals hardly noticeable secondary ornamentation in a form of longitudinal striae in between spirals. Under SEM, spirals rather high and flattened, sometimes slightly split, with reticulate ornamentation. Secondary ornamentation of thin ridges, more or less parallel to the elater longitudinal axis; places where ridges from different spirals meet are also sometimes reticulate. Spores yellow in mass, pale yellow in TL, usually subglobose, but sometimes of somewhat irregular shape, warted, (9.3–) 10.0–10.7 (–11.1) μm diam. (Mean = 10.34; SD = 0.39; n = 52). Under SEM, spore ornamentation pilate: columnar structure rather high, heads of caputs densely grouped. Secondary ornamentation usually absent, sometimes presented by regions of minute verrucae, visible only with high-definition SEM. Plasmodium not observed.

Habitat: rotten wood (in the field).

Distribution: Russia: Far East (Primorye Territory).

Holotype: RUSSIA, Primorye Territory, Chuguyevka District, Oblachnaya Mountain, Ussuri taiga, 43.69808°N 134.20029°E, August 2015, leg. E.A. Antonov, on rotten wood (MYX 6346).

Notes: the diagnostic features of this species are relatively small brown sporangia on short stalks, extremely long elaters, which are apparently more than 1 mm long, with short tips about 16 μm, clearly visible rough spiral bands, and distinctive secondary ornamentation of numerous longitudinal ridges, sometimes forming small reticulate areas.

This species is a part of the complex of closely related species – *Trichia nubila*, *T. acetocorticola*, and *T. pinicola* – that have almost identical ornamentation of inner peridium, spores, and capillitium and overlapping ranges of spore sizes. Nevertheless, these species can be reliably distinguished by a unique combination of only two traits: 1) elater width (medium or

Table 2. Diagnostic features of *Trichia nubila*, *T. acetocorticola*, *T. acetocorticola* var. *aggregata* and *T. pinicola*.

Taxon	*Trichia nubila*	*T. acetocorticola*	*T. acetocorticola* var. *aggregata*	*T. pinicola*
Elater length (μm)	400–1800 (fragments)	370–4020 (fragments)	440–980	180–712
Elater width (μm)	6.1–6.8	6.4–7.8	6.3–7.1	4.6–5.4
Length of elater tips (μm)	16 (13–18)	30 (19–41)	44 (28–53)	46 (27–62)
Substrate	rotten wood	bark of living trees	bark of living trees	bark of living trees
Substrate pH	NA (field specimen)	4.2–5.2	4.0	4.0–4.2

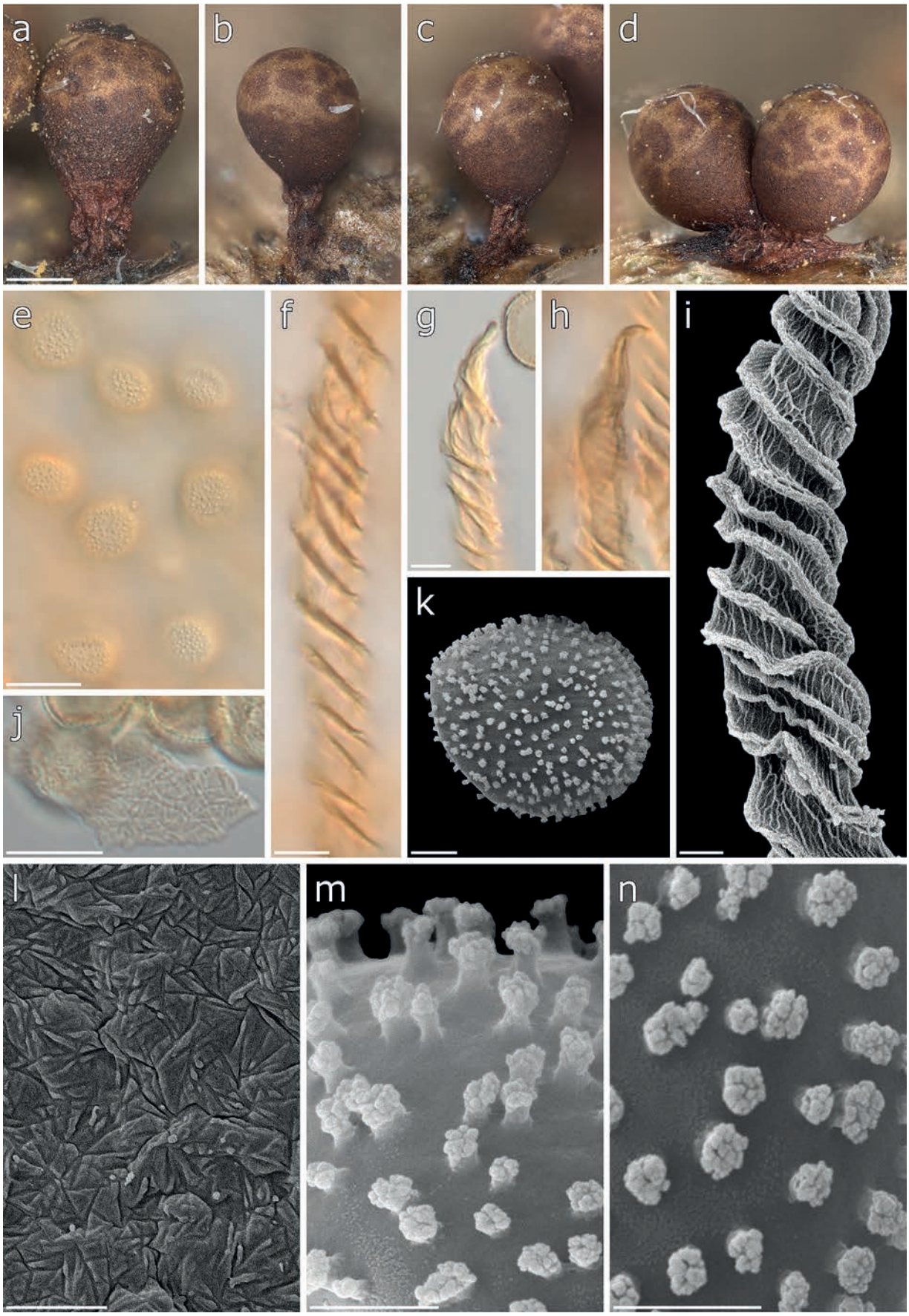

Figure 7 *Trichia nubila* **sp. nov.** (a–n): a–d – sporocarps, e – spores (LM), f, g, h – elaters (LM), i – elater (SEM), j – peridium (LM), k – spore (SEM), l – inner peridium (SEM), m, n – spore ornamentation. a–n – from MYX 6346 (holotype). Scale bars: a–d – 300 μm, e, j – 10 μm, f, g, h, l – 5 μm, i, k – 2 μm, m, n – 1 μm

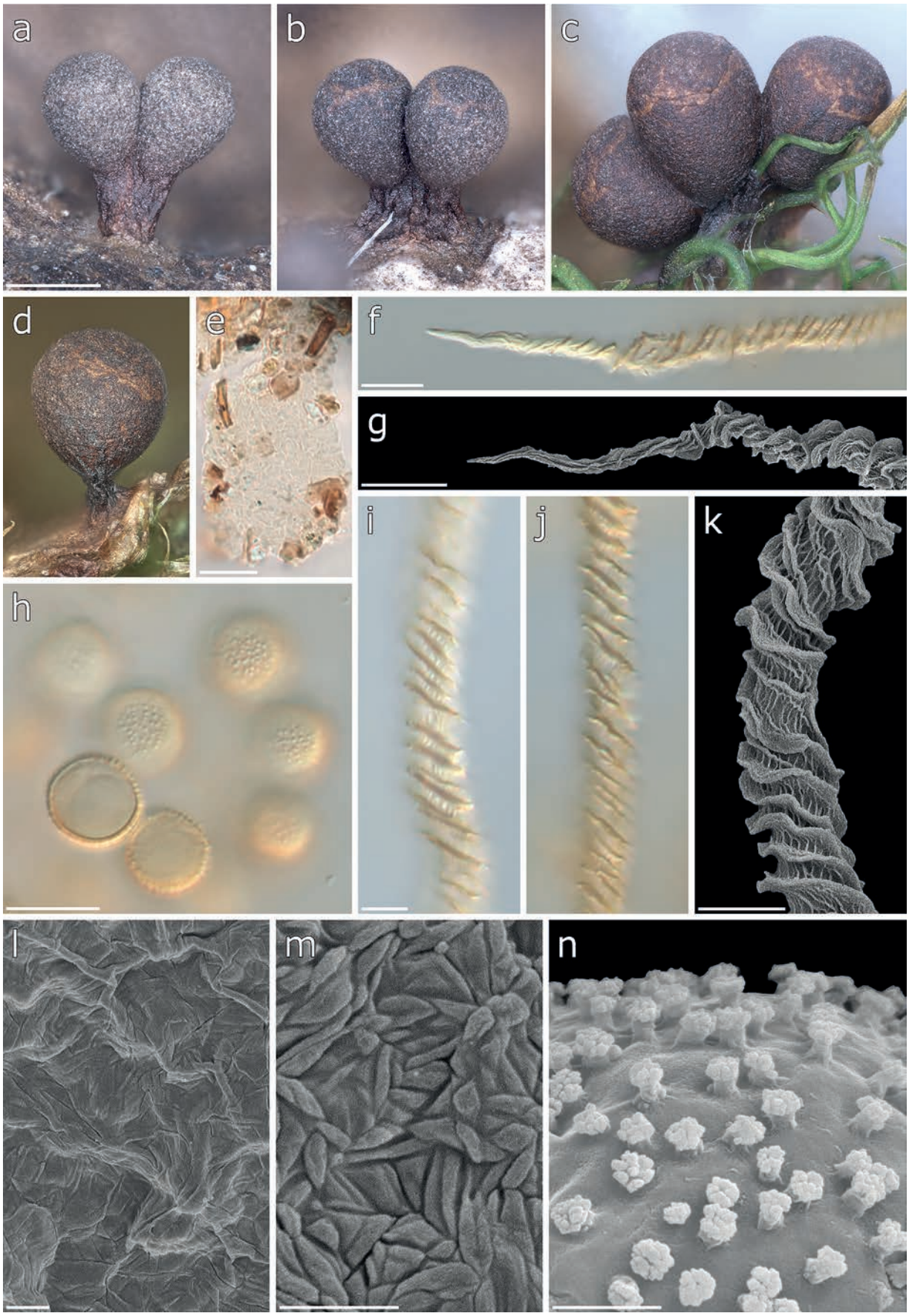

Figure 8 *Trichia acetocorticola* var. *acetocorticola* **sp. nov.** (a–n): a–d – sporocarps, e – peridium (LM), f, g – tips of elaters (LM and SEM), h – spores (LM), i, j, k – elaters (LM and SEM), l, m – inner peridium (SEM), n – spore ornamentation (SEM). a, b, f, j, l – from MYX 10073 (holotype), c, d, e, g, h, i, k, m, n – from MYX 8283. Scale bars: a–d – 500 μm, e, f, g, h – 10 μm, i, j, k – 5 μm, l, m – 3 μm, n – 1 μm

large) and 2) length of elater tips (short or medium). Additional characteristics are also 3) ecological niche (rotten wood or bark of living trees with high acidity) and 4) elater length (long or very long) (see Table 2).

A rare species *Trichia nodosa* G. Moreno, D.W. Mitch., W.CA. Rosing & S.L. Stephenson, described from Singapore, may also be included in this species complex (*acetocorticola–nubila–pinicola*) due to its distinctive rough ornamentation of capillitium. However, it differs well from all three mentioned species in spores 7–8 µm diam., that are very small in terms of the genus *Trichia*, and, in addition, in elaters typically branched with nodular swellings and prominent spines (Moreno et al. 2013).

Trichia acetocorticola **var.** ***acetocorticola*** Bortnikov **sp. nov.** Figs 8, S6–S11

MycoBank: 848473.

Etymology: refers to the substrate preference for acidic bark (pH 4–5).

Description. Sporocarps stalked sporangia, in sparse or dense groups, 920–1460 µm high. Stalk tuberculous, largely furrowed, 260–540 µm high, 27–37 % TH, greyish-brown, evenly colored, filled with refuse matter. Sporotheca single or in groups of 2–3 on a common stalk, obovate, 520–690 µm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with a lot of small straight striae, intersecting at different angles and forming a mosaic pattern. Outer layer made of granular matter, silvery grey to reddish-dark-brown, external deposits practically monolithic, without any distinct peridium plates. Dehiscence by preformed lines or irregular. Capillitium consisting of rusty yellow elaters, pale yellow in TL, simple, extremely long: presumably 600–900 µm or more (most of the elaters were ripped from one or two sides because of the great length; 17 fragments observed were 370–4020 (950 avg.) µm long; 5 intact elaters observed were 430–1800 (590 avg.) µm long). Elaters (6.1–) 6.4–7.8 (–8.6) µm (n = 35) wide. Tips of elaters medium, ca. 30 (19–41) µm long, gradually tapering to a very pointed end. Capillitial threads decorated with unevenly arranged, thin, left-handed spiral bands with ca. 7–10 turns per 20 µm. By LM, spirals with uneven edges, somewhat sharped. Under SEM, spirals quite high and blunt, sometimes slightly split or with almost reticulate ornamentation. Secondary ornamentation of thin ridges, more or less parallel to the elater longitudinal axis; places where ridges from different spirals meet are also sometimes reticulate. Spores yellow in mass, pale yellow in TL, usually subglobose, but sometimes of somewhat irregular shape, (8.4–) 9.5–11.1 (–12.8) µm diam. (Mean = 10.27; SD = 0.81; n = 158). Under SEM, spore ornamentation pilate: pila distanced from one another. Secondary ornamentation noticeable, represented by minute verrucae, arranged quite densely in some areas. Plasmodium not observed.

Habitat: bark of living trees (*Pinus koraiensis* Siebold & Zucc., *Betula schmidtii* Regel) with pH ca. 4.2–5.2 (in moist chambers).

Distribution: Russia: Far East (Primorye Territory).

Holotype: RUSSIA, Primorye Territory, Kedrovaya Pad Nature Reserve, dry oak-wood with *Fraxinus rhynchophylla* and *Ulmus japonica*, 43.128167°N 131.436500°E, 22.11.2017, leg. F.M. Bortnikov, on the bark of living *Betula schmidtii* (in moist chamber), pH 5.16 (MYX 10073).

Other studied specimens (paratypes): see table S1.

Notes: the main diagnostic features of this species are typically silvery gray sporangia with very dense deposits of the outer peridium, which hide the dehiscence lines, so that they seem very vague or not visible at all under the continuous layer. Similar deposits were previously observed in representatives of other genera of bright-spored myxomycetes: *Licea* Schrad., *Dianema* Rex, and *Perichaena* Fr. It has to be noted that crystalline deposits of *Perichaena* spp. are not usually formed if sporulation took place in moist chambers rather than in nature (Gilert 1990). However, specimens of *T. acetocorticola* were collected exactly from moist chambers.

The ecology of this species is also specific: it occurs on the bark of living trees with high acidity, with pH of about 4.0–5.0.

This species is a part of the complex of closely related species: *T. nubila*, *T. acetocorticola*, *T. pinicola*. See notes on *T. nubila*.

Trichia acetocorticola **var.** ***aggregata*** Bortnikov & Bortnikova **var. nov.** Figs 9, S6–S11

MycoBank: 848474.

Etymology: this variety is named so because of the very dense colonies, which are not typical of *Trichia botrytis* complex.

Description. Sporocarps stalked sporangia, in large, densely packed groups, 860–1290 µm high. Stalk tuberculous, largely furrowed, 320–540 µm high, 36–43 % TH, greyish-brown, brown, sometimes with a reddish tint, evenly colored, filled with refuse matter. Sporotheca single or in small groups on a common stalk, from subglobose to obovate, 500–700 µm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with a lot of small straight striae, intersecting at different angles and forming a mosaic pattern. Outer layer made of grey-brown granular matter; peridium plates rounded, with contrasting boarders. Dehiscence by preformed lines. Capillitium consisting of yellow elaters, pale yellow in TL, usually simple, sometimes branched or even enclosed in circles, (440–) 450–830 (–980) µm (n = 17) long and (6.0–) 6.3–7.1 (–7.4) µm (n = 22) wide. Tips of elaters medium, ca. 44 (28–53) µm long, gradually tapering to a pointed end. Capillitial threads decorated with unevenly arranged, thin, left-handed spiral bands with ca. 6–7 turns per 20 µm. By LM, spirals with uneven edges, somewhat sharped. Under SEM, spirals quite high and blunt, sometimes slightly split or with almost reticulate ornamentation. Secondary ornamentation of thin ridges, more or less parallel to the elater longitudinal axis; places where ridges from different spirals meet are also sometimes reticulate. Spores yellow in mass, pale yellow in TL, usually subglobose, but sometimes of somewhat irregular shape, warted, (9.3–) 10.0–11.0 (–11.7) µm diam. (Mean = 10.49; SD = 0.52; n = 104). Under SEM, spore ornamentation pilate: pila distanced from one another, without any bridges. Secondary ornamentation absent. Plasmodium not observed.

Habitat: bark of living trees (*Pinus massoniana* Lamb.), pH 4.02 (in moist chambers).

Distribution: Vietnam (Cao Bang Province).

Holotype: VIETNAM, Cao Bang Province, Phia Oac – Phia Den National Park, artificial plantation of *Pinus massoniana* and *Musa* spp., 22.57472°N 105.87173°E, April 2019, leg. Yu.K. Novozhilov, N.A. Bortnikova, on the bark of living *Pinus massoniana* (in moist chamber), pH 4.02 (LE 324624).

Notes: this variety differs from the typical one in the more densely crowded colonies, pronounced peridium plates, and longer elater tips (44 µm avg. compared to 30 µm).

However, considering the extreme similarity in other features – size and color of sporangia, ornamentation of the inner peridium, spore size, and elater ornamentation – as well as identical ecology and close phylogenetic position, we believe that treating this taxon as a separate species is not yet sufficiently justified. Molecular differences between two varieties are reduced to a large number of substitutions in the nrSSU sequence and a few indels in nrSSU and mtSSU gene fragments.

This species is a part of the complex of closely related species: *Trichia nubila*, *T. acetocorticola*, *T. pinicola*. See notes on *T. nubila*.

Trichia pinicola Bortnikov, Bortnikova & Novozhilov **sp. nov.** Figs 10, S6–S11

MycoBank: 848475.

Etymology: refers to the substrate specificity – all specimens were obtained from *Pinus massoniana* with acidic bark.

Description. Sporocarps stalked sporangia, scattered, 800–1560 µm high. Stalk largely furrowed, 230–960 µm, 27–61 % TH, brown to black, sometimes lighter near the base, filled with refuse matter. Sporotheca single or by two on a common stalk, obovate to subglobose, 450–700 µm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with a lot of small straight striae, intersecting at different angles and forming a mosaic pattern; sometimes polygonal areas between striae decorated with small invaginations. Outer layer

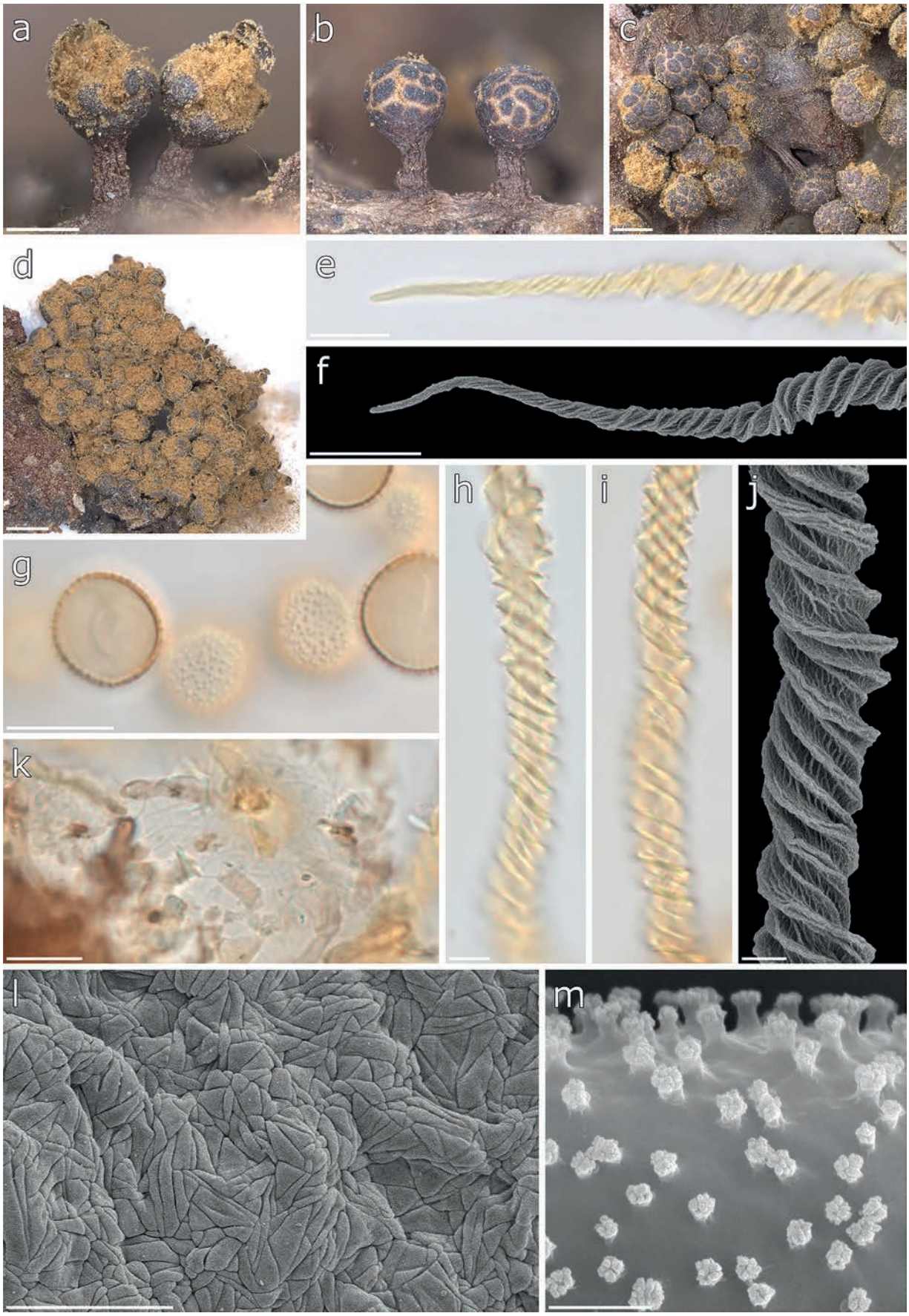

Figure 9 *Trichia acetocorticola* var. *aggregata* **var. nov.** (a–m): a–d – sporocarps, e, f – tips of elaters (LM and SEM), g – spores (LM), h, i, j – elaters (LM and SEM), k – peridium (LM), l – inner peridium (SEM), m – spore ornamentation (SEM). a–m – from LE 324624 (**holotype**). Scale bars: d – 1000 μm, a, b, c – 500 μm, e, f, g, k, l – 10 μm, h, i – 5 μm, j – 2 μm, m – 1 μm

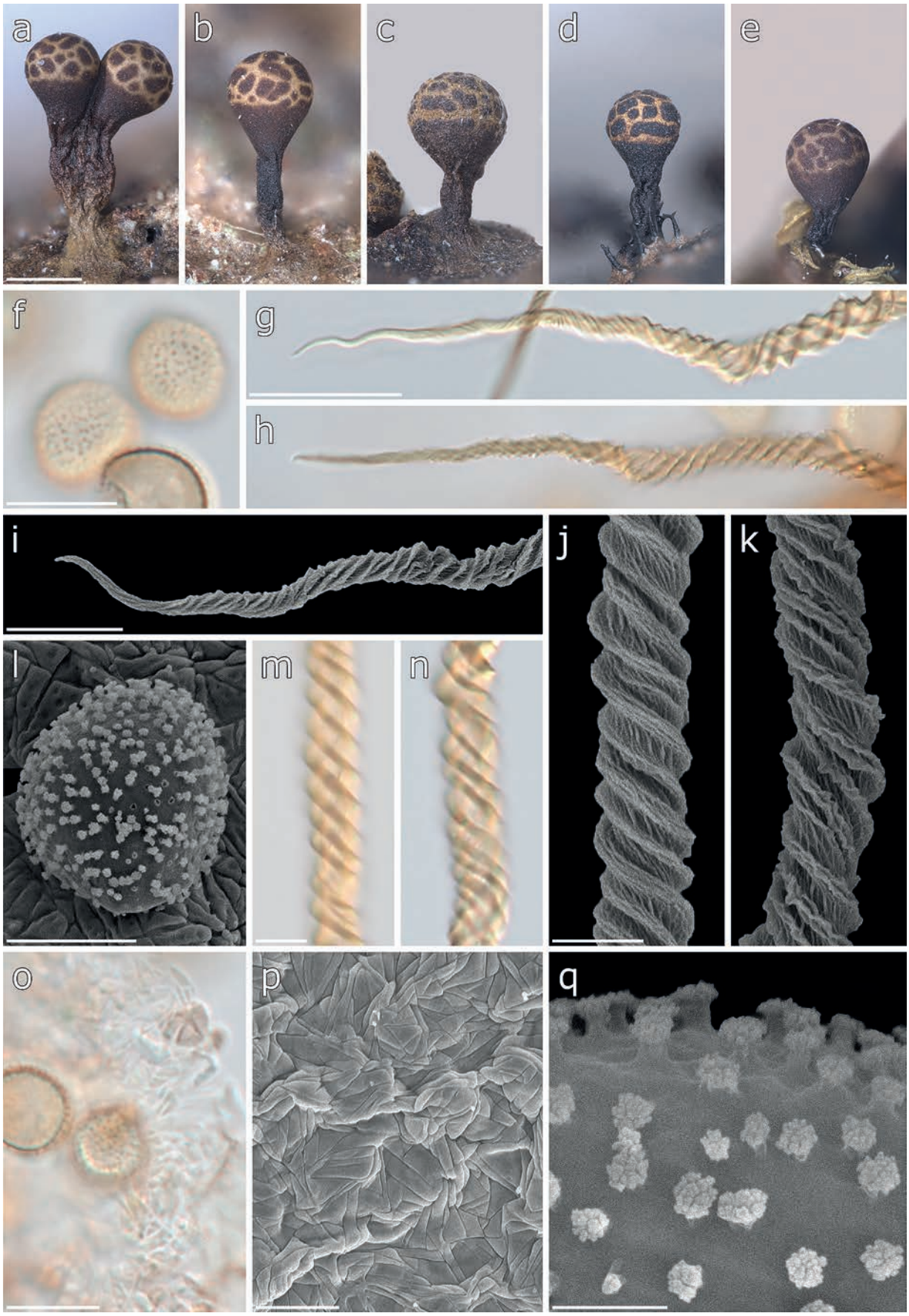

Figure 10 *Trichia pinicola* **sp. nov.** (a–q): a–e – sporocarps, f – spores (LM), g, h, i – tips of elaters (LM and SEM), j, k – elaters (SEM), l – inner peridium and spore (SEM), m, n – elaters (LM), o – peridium (LM), p – inner peridium (SEM), q – spore ornamentation (SEM). a, f, o, p – from LE 324628 (**holotype**), b, j, l, m, q – from LE 324629, c, i – from LE 324670, d – from LE 324613, e, g, n – from LE 324671, h, k – from LE 324673. Scale bars: a–e – 500 μm, g, h – 20 μm, f, i, o – 10 μm, l, m, n, p – 5 μm, j, k – 3 μm, q – 1 μm

made of granular matter, greyish-brown to reddish-dark-brown; peridium plates rounded, conspicuous. Dehiscence by preformed lines. Capillitium consisting of yellow to rusty yellow or brownish-yellow elaters, pale yellow in TL, (180–) 312–513 (–712) μm (n = 186) long and (3.8–) 4.6–5.4 (–6.0) μm (n = 92) wide. Tips of elaters medium, ca. 46 (27–62) μm long, gradually tapering to a pointed end. Capillitial threads decorated with left-handed spiral bands, quite unevenly arranged, with ca. 8–10 (–12) turns per 20 μm. By LM, spirals somewhat sharped, occasionally longitudinally split. Oil immersion reveals hardly noticeable secondary ornamentation in a form of longitudinal striae in between spirals. Under SEM, spirals quite low, either smooth with fibrous surface or fimbriate and longitudinally split. Secondary ornamentation of thin ridges, more or less parallel to the elater longitudinal axis. Spores light yellow or yellow in mass, pale yellow in TL, usually subglobose, but sometimes of somewhat irregular shape, (8.3–) 9.3–10.8 (–12.5) μm diam. (Mean = 10.05; SD = 0.72; n = 467). Under SEM, spore ornamentation pilate: pila separate, without any bridges. Secondary ornamentation absent. Plasmodium not observed.

Habitat: bark of living *Pinus massoniana* with pH 4.0–4.2 (in moist chambers).

Distribution: Vietnam (Cao Bang Province).

Holotype: VIETNAM, Cao Bang Province, Phia Oac – Phia Den National Park, artificial plantation of *Pinus massoniana* and *Musa* spp., 22.57472°N 105.87173°E, April 2019, leg. Yu.K. Novozhilov, N.A. Bortnikova, on the bark of living *Pinus massoniana* (in moist chamber), pH 4.00 (LE 324628).

Other studied specimens (paratypes): see table S1.

Notes: the main diagnostic features of this species are typically dark brown sporangia with clearly visible, contrasting peridium plates, rather long (usually about 312–513 μm) and not very wide (about 4.6–5.4 μm) elaters with gradually tapering tips of medium length (about 46 μm), and ecological preference for the bark of living trees with high acidity (about 4.0–4.2 pH, according to our data).

This species is a part of the complex of closely related species: *Trichia nubila*, *T. acetocorticola*, *T. pinicola*. See notes on *T. nubila*.

Trichia papillata Adamonytė Figs 11, S6–S11

Description. Sporocarps stalked sporangia, solitary or in scattered groups, 830–1200 μm high. Stalk slightly tuberculous or furrowed, comparatively long, 450–720 μm high, 54–64 % TH, dark brown to almost black, sometimes lighter near the base, filled with refuse matter. Sporotheca single, obovate to subglobose, slightly elongated in the lower part, 260–400 μm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with irregular lines made up of warts, areas between lines finely warted in TL. Under SEM, ornamentation more chaotic, composed of various rods and warts. Outer layer made of granular material, brown to dark brown. Peridium plates large, polygonal, usually no more than 7 on a visible part of sporotheca, with prominent, almost black papillae and distinct margins. Dehiscence by preformed lines. Capillitium consisting of yellow to bright yellow elaters, pale yellow in TL, always simple, (205–) 305–672 (–907) μm (n = 20) long and (3.5–) 3.9–4.7 (–4.9) μm (n = 11) wide. Capillitial threads decorated with left-handed spiral bands, quite evenly arranged, ca. 11 bands per 20 μm. In TL, spirals warted or even spiny. Under SEM, spiral bands undoubtedly decorated only with sometimes slightly elongated warts and conspicuously longitudinally split. Secondary ornamentation absent. Tips of elaters short to medium, ca. 18 (11–22) μm long, gradually tapering to a pointed end. Spores bright yellow in mass, pale yellow in TL, usually subglobose, but sometimes of somewhat irregular shape, unevenly warted, small, (7.8–) 8.3–9.0 (–9.4) μm diam. (Mean = 8.64; SD = 0.36; n = 50). Under SEM, spore ornamentation pilate: pila distanced from one another, without bridges between caputs. Secondary ornamentation absent. Plasmodium not observed.

Habitat: leaf litter of *Quercus robur* L., pH 6.07 (in moist chamber).

Distribution: Russia: European part (Volgograd Region).

Studied specimen: see table S1.

Notes: *T. papillata* is the closest species to *T. musicola* based on the phylogenetic analyses – the clade has maximum supports by both ML and BI (Fig. 1). Despite being unambiguously different in general habit (*T. musicola* lacks any papillae and has light yellow sporotheca), these species have a lot of micromorphological features in common: spores are ornamented with pila, spiral bands are decorated with small warts, inner peridium is warted, usually with a pattern of straight lines, visible in TL. However, spores of *T. papillata* are smaller (8.3–9.0 vs 9.7–10.9 μm), without secondary ornamentation, pila are separate, without any thread-like connections, stubbier, with smoother caputs, and capillitial threads are thinner (3.9–4.7 vs 4.7–5.6 μm) and shorter (306–672 vs 180–1000 μm).

Studied specimen is the second documented record of *T. papillata* in Russia. The first one was found in Moscow on coniferous litter during the experiment with moist chamber cultures (Gmoshinskiy et al. 2019). It is characterized by slightly thinner elaters (3.0–3.5 vs 3.9–4.7 μm) with occasional swellings up to 8–10 wide, which might be a result of aberrant development or endoparasite as in case of nivicolous *Lamproderma* Rostaf. (Yajima et al. 2013) or *T. munda* (discussed below).

Although the holotype was found on leporoid dung and paratypes – on cervoid dung, other specimens of *T. papillata* mentioned in the literature were discovered on plant litter (Liu et al. 2007, Gmoshinskiy et al. 2019) and wood (Novozhilov et al. 2017a), which suggests the facultative nature of this species preference for dung substrate (if all these records are truly conspecific). Our specimen from Volgograd Region corresponds to this thought. Nevertheless, it differs from the holotype in slightly smaller spores (8.3–9.0 vs 9.0–10.3 μm), somewhat thinner capillitial threads (3.9–4.7 vs 4.5–5.0), and almost complete absence of capillitium branches. It is worth mentioning the specimen from Taiwan (Liu et al. 2007), which is characterized by spores of similar diameter (8.0–8.5 vs. 8.3–9.0 μm), narrower elaters (3.0–3.5 vs. 3.9–4.7 μm), and abundant peridium plates in comparison with our specimen. Unfortunately, there is no information on the length of whole elaters or their tips in any source. Although all the specimens discussed are still quite similar, their conspecificity is to be clarified in future.

Trichia musicola Bortnikov & Bortnikova **sp. nov.** Figs 12, S6–S11

MycoBank: 848469.

Etymology: refers to the substrate, leaf litter of *Musa* spp., where all three specimens known at the time were found.

Description. Sporocarps stalked sporangia, scattered, 780–1600 μm high. Stalk finely furrowed, rather long, 400–920 μm high, 51–58 % TH, dark brown to blackish-brown, often lighter in the upper part, filled with refuse matter. Sporotheca single or in groups of 2–3 on a common stalk, obovate, subglobose or flattened, so width (430–620 μm) exceeds height (380–680 μm). Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with warts and straight striae, intersecting at different angles. Under SEM, warts forming a unique ornamentation of incomplete ridges of varying height, so that the pattern can be compared to mud oozing from under the car wheels in bad weather. Outer layer made of granular matter, light yellow to brown-yellow or orange-brown. Peridium plates not contrasting due to the gradual thinning of deposits. Dehiscence by preformed lines. Capillitium consisting of yellow elaters, pale yellow in TL, long, usually simple, (170–) 180–1000 (–2100) μm (n = 92) long and (4.3–) 4.7–5.6 (–6.0) μm (n = 37) wide. Capillitial threads decorated with evenly arranged left-handed spiral bands, ca. 10–11 (–12) turns per 20 μm. In TM, spirals scabrous or finely warted. Under SEM, spirals ornamented with small warts. Secondary ornamentation not prominent, spaces between turns finely fibrous to smooth. Tips of elaters rather short, ca. 14 (8–19) μm long, almost always with subterminal swellings. Spores yellow in mass, pale yellow in TL, sometimes subglobose, but usually of somewhat irregular shape, warted, (9.0–) 9.7–10.9 (–12.1) μm diam. (Mean = 10.31; SD = 0.58; n = 205). Under SEM,

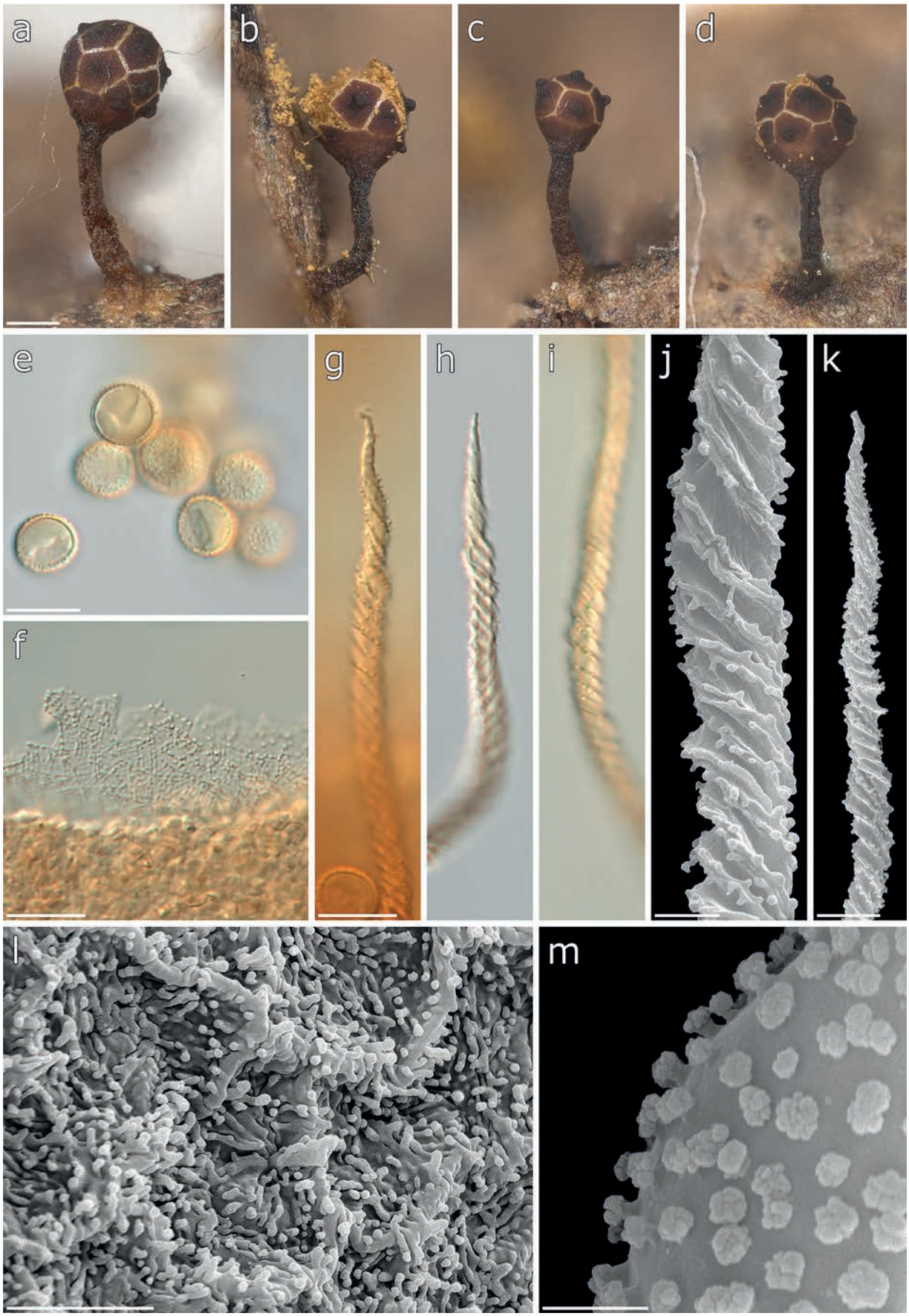

Figure 11 *Trichia papillata* Adamonyte (a–m): a–d – sporocarps, e – spores (LM), f – peridium (LM), g, h, i – elaters (LM), j, k – elaters (SEM), l – inner peridium (SEM), m – spore ornamentation (SEM). a–m – from LE 277823. Scale bars: a–d – 200 μm, e–i – 10 μm, k, l – 5 μm, j – 2 μm, m – 1 μm

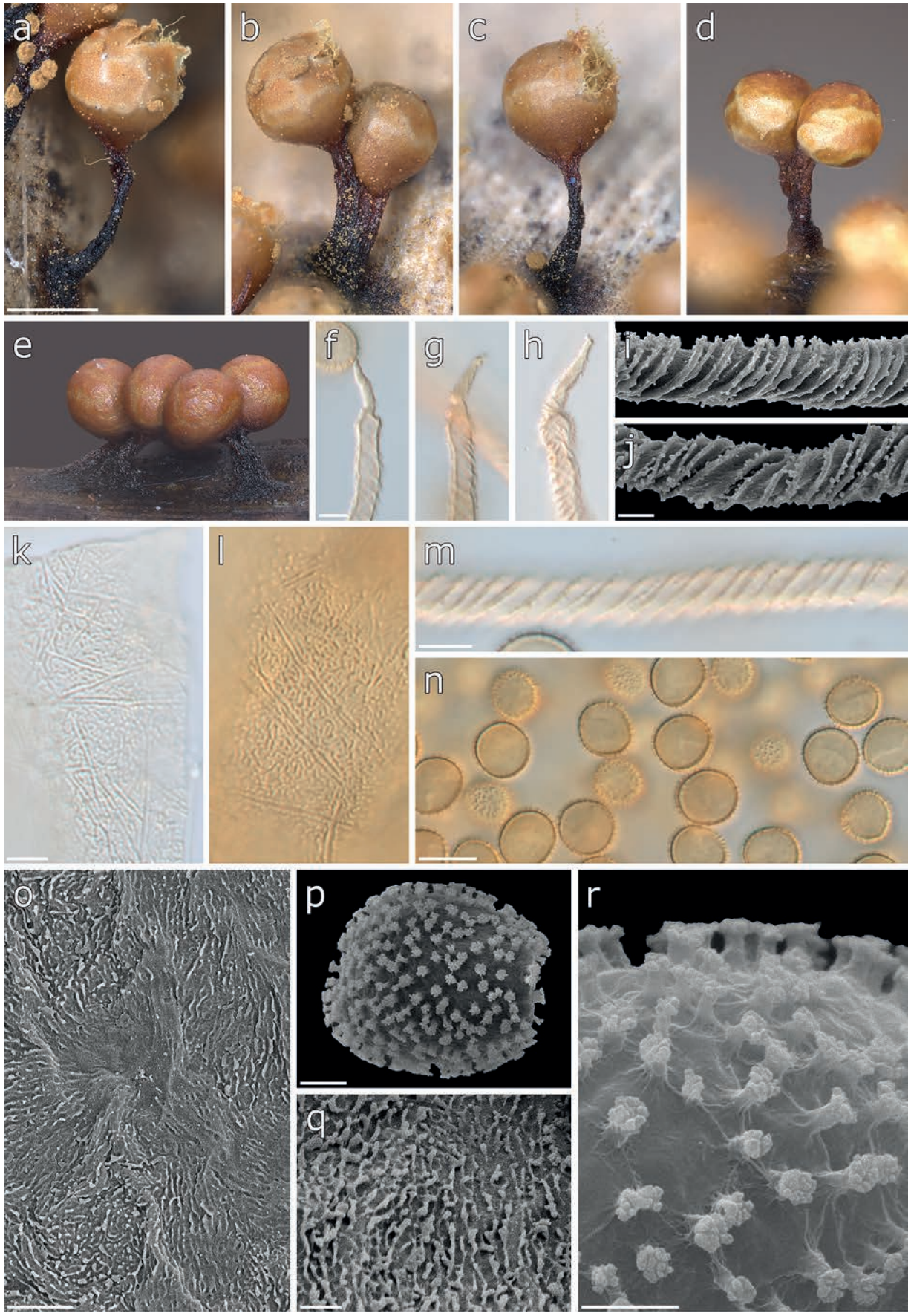

Figure 12 *Trichia musicola* **sp. nov.** (a–r): a–e – sporocarps, f–h – tips of elaters (LM), i, j – elaters (SEM), k, l – peridium (LM), m – elater (LM), n – spores (LM), o, q – inner peridium (SEM), p, r – spore ornamentation (SEM). a, b, c, i, o, p – from LE 317635, d, e, f, g, h, j, k, l, m, n, q, r – from LE 326224 (**holotype**). Scale bars: a–e – 500 μm, n – 10 μm, f, g, h, k, l, m, o – 5 μm, i, j, p, q – 2 μm, r – 1 μm

spore ornamentation pilate: columnar structure high; heads of caputs grouped, but rather distant; sometimes nearby pila are joined by thin bridges. Secondary ornamentation of fibrous structures radiating from the base of every pilum. Plasmodium not observed.

Habitat: leaf litter (including aerial litter) of *Musa* spp., pH 7.3–8.3 (in the field and moist chambers).

Distribution: Vietnam (Cao Bang, Lam Dong, and Phu Tho Provinces).

Holotype: VIETNAM, Cao Bang Province, Phia Oac – Phia Den National Park, closed evergreen tropical monsoon submontane broad-leaved forest, 22.591573°N 105.890080°E, 09.10.2019, leg. Yu.K. Novozhilov, O.N. Shchepin, on air litter of *Musa* sp. in the field (LE 326224).

Other studied specimens (paratypes): see table S1.

Notes: the main diagnostic features of the new species are light yellow sporocarps with low-contrast peridium dehiscence lines, warted inner peridium, rather sparse and rough pilate spore ornamentation, long capillitial threads (up to 2 mm) decorated with left-handed spirals with small warts, and short elater tips with small subterminal swellings.

A noticeable variation in elater length was observed: specimen LE 326224 had elaters 200–450 µm long avg., LE F-348754 – 300–730 µm, and LE 317635 – 560–1500 µm. However, the average ranges and even more so the maximum values overlap, and ornamentation of elaters, shape of their tips, and other details of micro- and macromorphology of these specimens coincide; the nrSSU and EF1α sequences are fully identical, so there is no doubt about their conspecificity.

Trichia musicola is characterized by a very distinct morphology and can hardly be confused with any other species. Its micromorphology is similar to that of *Trichia papillata* and *Hemitrichia velutina* Nann.-Bremek. & Y. Yamam. that also have spiral bands decorated with small warts (Nannenga-Bremekamp & Yamamoto 1986, Adamonytė 2003). *T. papillata*, described from Lithuania (Adamonytė 2003) and also found in Southeast Asia (Liu et al. 2007), has features in common with *T. musicola*, namely warted inner peridium and short elater tips, but has significant differences, too. First, SEM studies show that pila ornamenting spores of *T. papillata* are stumpy, with rounded heads of caputs and no fibrous structures radiating from the base of pila. Second, *T. papillata* has a remarkably characteristic outer peridium of dark polygonal plates with very contrasting margins and one papilla in the center of each plate. *Trichia musicola*, on the other hand, has a very light outer peridium, with smooth, gradual plate margins and without any papillae. Therefore, these two species are definitely different, although their close relationship is assumed based on similar morphological features and reliably confirmed by the results of phylogenetic analysis (Fig. 1).

Hemitrichia velutina, described from Japan, is similar to *Trichia musicola* in that it has elaters with spiral bands decorated with small warts and short tips with small subterminal swellings (Nannenga-Bremekamp & Yamamoto 1986, Liu et al. 2002a). This species, however, can be easily distinguished by the following characteristics: firstly, the capillitium is branched, although quite scantily, which is the reason of placing this species in the genus *Hemitrichia* s. l.; secondly, the outer peridium is dark and comprises more plates; thirdly, the spores are smaller, about 8 µm compared with at least 9 µm in case of *T. musicola*. Finally, *H. velutina* is an acidophilic corticolous species whose specimens were gathered from moist chambers with bark of living *Chamaecyparis obtusa* (Siebold & Zucc.) Endl (Nannenga-Bremekamp & Yamamoto 1986) and *Cryptomeria japonica* (Thunb. ex L.f.) D. Don (Liu et al. 2002a), whose pH values are 3.2–4.1 and 2.8–4.4, respectively, according to the literature (Takahashi 2014), whereas *T. musicola* inhabits *Musa* spp. leaf litter with pH about 7–8.

Of the three currently known specimens of *Trichia musicola*, two were collected in the field from banana leaf litter (*Musa* spp.). The third was obtained in a moist chamber with leaf axils of *Musa* sp. and pH 8.25. This allows us to assume that leaf litter of *Musa* spp. is a common microhabitat of this species. Also, in other moist chamber experiments with banana litter, the median pH value of this substrate was 7.3.

Trichia flavicoma (Lister) Ing Figs 13, S6–S11

Description. Sporocarps stalked sporangia, solitary, (128–) 171–320 (–462) µm high (n = 33). Stalk rough, finely tuberculous, (16–) 34–109 (–174) µm high (n = 33), 10–45 % TH (19–37 % avg.), dark orange-brown to almost black, filled with refuse matter. Sporotheca always single, from subglobose to obovate, (88–) 115–206 (–319) µm wide, H/W ratio 1.09 avg. Peridium two-layered. Inner layer thin, membranous, hyaline, finely warted, decorated with thin straight striae, intersecting at various angles. Under SEM, ornamentation composed of small rounded warts, solitary or uniting in short ridges. Outer layer made of granular matter, yellow-brown to dark brown. Dehiscence by preformed lines. Dehiscence lines usually quite wide and contrast. Capillitium consisting of yellow or dull yellow elaters, pale yellow in TL, simple, (150–) 200–370 (–540) µm (n = 81) long and (2.6–) 3.0–3.6 (–4.1) µm (n = 84) wide, elastic and often sinuous. Capillitial threads decorated with left-handed spiral bands, rather densely and evenly arranged, ca. (6–) 8–14 turns per 20 µm. Under SEM, spiral bands of elaters smooth, without conspicuous secondary ornamentation. Tips of elaters short or medium, ca. 17 (7–24) µm long, tapering very gradually, with their bases hard to distinguish; often with blunt, widened, aberrant ends in the case of studied specimens. Spores bright yellow in mass, pale yellow in TL, sometimes subglobose, but usually of somewhat irregular shape, warted; (8.7–) 9.1–10.0 (–11.3) µm diam. (Mean = 9.55; SD = 0.47; n = 65) [specimen from Vietnam], (9.9–) 10.7–12.2 (–13.5) µm diam. (Mean = 11.43; SD = 0.73; n = 272) [specimens from the Russian Far East]. Under SEM, ornamentation pilate: columnar structure rather short, stumpy; caputs wide, with rounded, densely packed heads. High-definition SEM reveals secondary ornamentation of very small, simple, hemispheric warts, occasionally uniting in straight or slightly curved lines. Plasmodium is not observed.

Habitat: litter of *Juniperus davurica* Pall. and bark of living *J. davurica* in direct contact with litter on the ground; bark of unidentified deciduous tree, pH 6.21–7.08 (in moist chambers).

Distribution: Russia: Far East (Primorye Territory); Vietnam (Gia Lai Province).

Studied specimens: see table S1.

Notes: *Trichia flavicoma* was originally described from the United Kingdom in 1894 as *T. botrytis* var. *flavicoma* (Lister 1894). The species was described very briefly, and the only differences noted were minute sporangia (without any measurements), stalks 0.25 mm long, bright yellow elaters, and dead leaves as a substrate (in contrast to dead wood preferred by the typical var. *genuina* [≡ var. *botrytis*]). Based on the illustrations given (Plate LXII B, j, k, see Fig. 14), it can be concluded that the sporocarp size and the stalk length vary to some degree, for example, from about 195 to 315 µm in the case of the stalk (calculated using the illustration and the average length of 250 µm), and the outer peridium is usually divided in a large number of plates: 5 to 15–17 on a visible part of sporotheca. In 1967, Bruce Ing reported all the same differences for *T. botrytis* var. *flavicoma* as Lister had, but considered them sufficient to elevate the taxon to species rank (Ing 1967).

Our analysis included 6 specimens of *T. flavicoma*: one from Vietnam, obtained from the bark of an undetermined deciduous tree in moist chamber (pH 6.71), and five from one locality in the Russian Far East, obtained from litter and bark of *Juniperus davurica* in moist chambers, too (pH 6.21–7.08).

The studied specimens have smaller sporangia than it is indicated in the original description. Stalks are about 70 µm long avg. and do not exceed 174 µm. However, they coincide with the original description in other features, namely bright yellow spore mass and capillitium, a large number of peridium plates, association with ground litter (5 specimens). However, some of these five specimens from *Juniperus davurica* are indicted as corticolous according to the list of specimens (Table S1). The point is that this particular species of juniper is a creeping shrub, and the branches covered with bark thick enough to collect for the experiment with moist chamber cultures were in contact with the shrub's needle litter on the ground. Therefore, the specimen from Vietnam is the only one obtained specifically from bark.

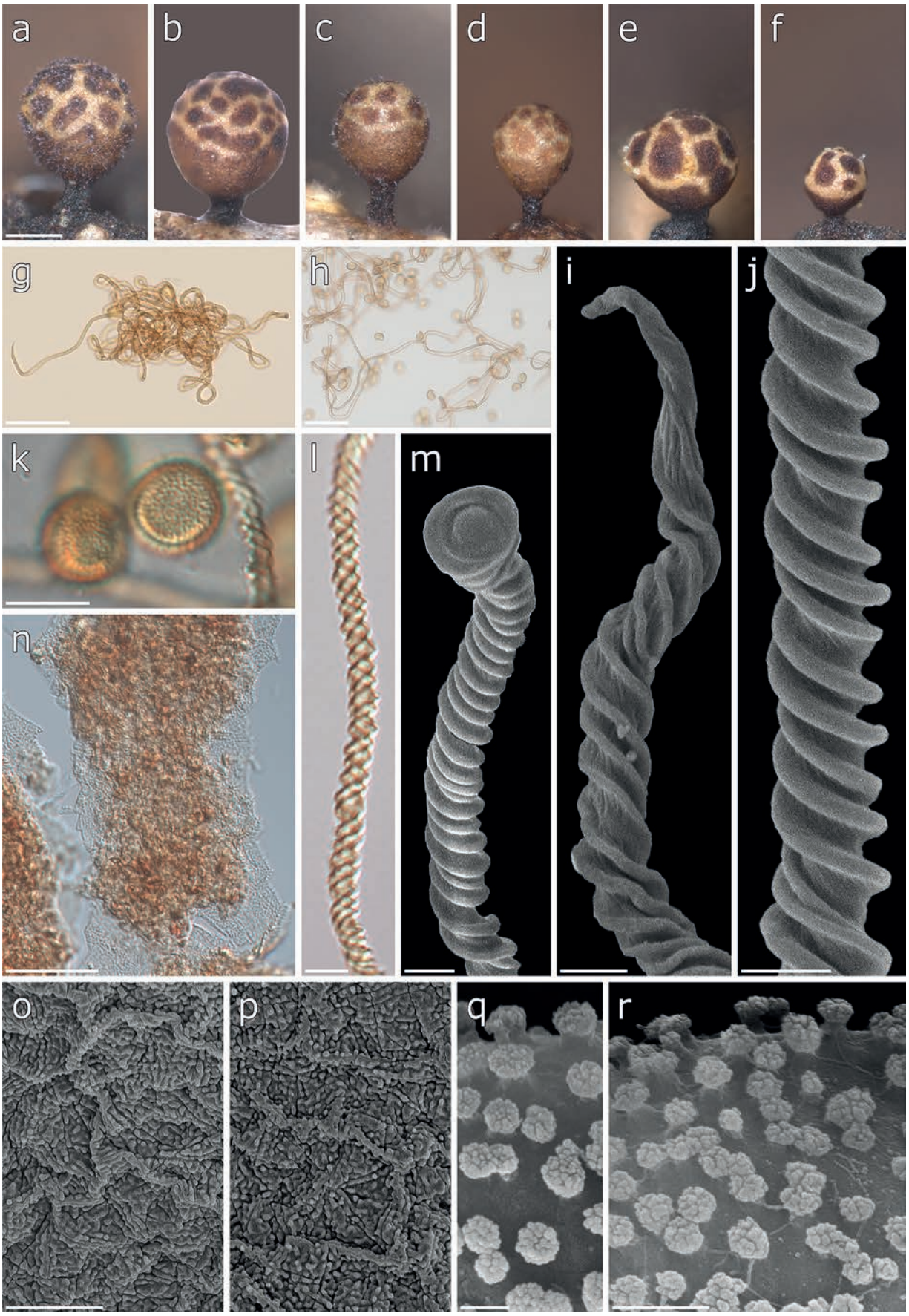

Figure 13 *Trichia flavicoma* (Lister) Ing (a–r): a–f – sporocarps, g, h – elaters (LM), i, m – normal and aberrant tips of elaters (SEM), j – elater ornamentation (SEM), k, l, n – spores, elater and peridium (LM), o, p – inner peridium (SEM), q, r – spore ornamentation (SEM). a, b, c, d, h, j, k, m, r – from MYX 21430, e, f, i, n, o, p, q – from LE F-348756 (Vietnam specimen), g – from MYX 21427, l – from MYX 21431. Scale bars: a–f – 100 μm, g, h – 50 μm, n – 20 μm, k – 10 μm, l, o, p – 5 μm, i, j, m – 2 μm, r – 1 μm, q – 0.5 μm

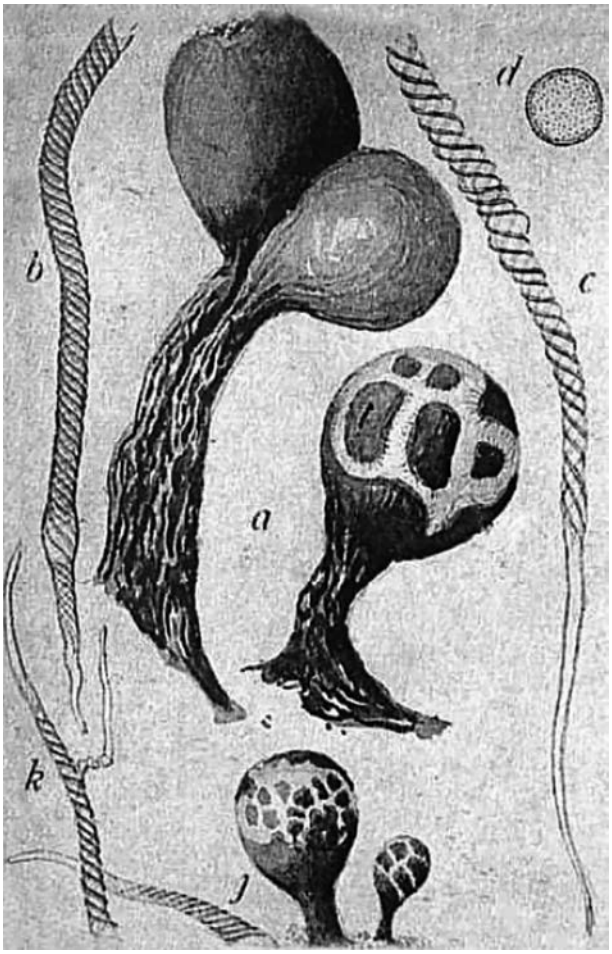

Figure 14 Plate LXII B from Lister, 1894. j, k – *Trichia botrytis* var. *flavicoma* (England). Source: Biblioteca Digital del Real Jardín Botánico: https://bibdigital.rjb.csic.es

We assume that specimens of *T. flavicoma* from the type region (United Kingdom) may have some morphological and/or molecular differences from our specimens, which have yet to be studied. However, since among all of the available specimens this very morphotype matched the description of *T. flavicoma* the most, we defined our collections as such.

The closest species to *T. flavicoma* is *T. titanica* (see further), and their most prominent differences lie in ecology (foliicolous vs corticolous) and elater length (long vs short) (see notes on *T. titanica*). Moreover, our observations suggest that *T. flavicoma* is a quite rare species in Primorye Territory (the Russian Far East) in contrast to *T. titanica*; in the Kedrovaya Pad Nature Reserve it has been found only in one locality out of more than 70 studied so far, and in the Sikhote-Alin Nature Reserve it has not been observed at all (Novozhilov et al. 2017b).

Trichia titanica Bortnikov, Bortnikova & Novozhilov **sp. nov.** Figs 15, S6–S11

MycoBank: 848467.

Etymology: epithet is ironic and refers to the smallest sporangia in the genus *Trichia*.

Description. Sporocarps stalked sporangia, rarely on very short stalks, only once completely sessile and pulvinate (Fig. 15f), not exceeding the radius of sporotheca, sometimes almost sessile, usually solitary or in very scattered groups, (79-) 121–207 (–345) μm high (n = 193). Stalk rough, finely tuberculous, (6–) 31–83 (–136) μm high (n = 193), 6–54 % TH (24–43 % avg.), translucent orangish-brown to almost black, filled with refuse matter. Sporotheca always single, globose to obovate, (58–) 78–127 (–197) μm wide, H/W ratio 1.05 avg. Peridium two-layered. Inner layer thin, membranous, hyaline, finely warted, decorated with thin straight striae, intersecting at different angles. SEM reveals ornamentation of small rounded warts, solitary or uniting in short ridges. Outer layer of granular matter, nut-brown or yellowish-brown to dark brown. Dehiscence by preformed lines via rupturing of the inner peridium (NB: absolutely dry sporangia from moist chambers almost never ruptured without mechanical disturbance). Dehiscence lines usually not very contrasting due to the dull color of spore mass and capillitium showing though the inner peridium. Capillitium consisting of yellow or dull yellow elaters, pale yellow in TL, usually simple, very short, more or less straight or sometimes curved, but never sinuous or intertwisted, (35–) 70–120 (–165) μm (n = 217) long and (2.6–) 3.1–3.8 (–4.2) μm (n = 137) wide. Tips of elaters short or medium, ca. 18 (11–28) μm long, tapering very gradually, with their bases hard to discern. Capillitial threads decorated with left-handed spirals, very densely and evenly arranged, ca. (14–) 15–18 (–19) bands per 20 μm; under SEM smooth, without any conspicuous secondary ornamentation. Spores dull yellow in mass, light yellow in TL, sometimes subglobose, usually of somewhat irregular shape, warted, (8.5–) 9.8–11.2 (–13.0) μm diam. (Mean = 10.50; SD = 0.71; n = 680). Under SEM, ornamentation pilate: columnar structure rather short, stumpy; heads of caputs rounded and tightly packed. High-definition SEM reveals secondary ornamentation of very small, simple, hemispheric verrucae, occasionally uniting in straight or S-curved lines. Plasmodium not observed.

Habitat: bark of living trees (*Chosenia arbutifolia* (Pall.) A.K. Skvortsov, *Juglans mandshurica* Maxim., *Kalopanax septemlobus* (Thunb. ex A.Murr.) Koidz., *Populus maximowiczii* A. Henry, *Quercus dentata* Thunb., *Q. mongolica* Fisch. ex Ledeb., *Tilia amurensis* Rupr.) with the median pH 6.9 (n = 54) (in moist chambers).

Distribution: Russia: Far East (Primorye Territory).

Holotype: RUSSIA, Primorye Territory, Kedrovaya Pad Nature Reserve, flood-plain forest with *Chosenia arbutifolia*, *Alnus hirsuta* (Spach) Rupr., and *Fraxinus* spp., 43.168930°N 131.505160°E, 14.03.2020, leg. F.M. Bortnikov, on the bark of living *Chosenia arbutifolia* (in moist chamber), pH 6.77 (MYX 21439).

Other studied specimens (paratypes): see table S1.

Notes: the main diagnostic features of the new species are minute fruiting bodies developing on the bark of living trees with high pH value (most often on *Chosenia arbutifolia*, *Kalopanax septemlobus*, and *Populus maximowiczii*), warted inner peridium, and short elaters ca. 70–120 μm long.

Trichia titanica was found in only 7 of 91 moist chambers with the bark of *Quercus mongolica* (one of the dominant tree species in the secondary forests of Primorye Territory), i.e., in 8 % of cases. We associate it with the fact that pH of *Quercus mongolica* bark is usually slightly lower than the optimal one for *T. titanica* (median pH value of oak bark is 6.28). On the bark of three tree species mentioned above, whose pH is consistently higher (median bark pH 6.77, 6.73, and 6.87, respectively), this species occurs much more frequently (at least in 23, 50, and 44 % of cases, respectively), and in fact may be even more common, and here is why.

Trichia titanica is the smallest species of *Trichia* known to date: sporocarps reach an average height of only 164 μm. In addition, sporangia are almost always scattered and never form abundant dense colonies. Finally, because of the dull yellow color of the spores, dehiscence lines on the peridium are not very bright and contrasting, and the whole sporangium is difficult to notice on bark of similar color. Apparently, these reasons explain the fact that this species has not been discovered so far, and even if it has been, it may have been recorded as aberrant forms of sporangia of other species of *Trichia* poorly developed in moist chambers. However, according to our observations, *T. titanica* is a widespread species often found in the Russian Far East. If researcher notices sporocarps of *T. titanica* even once, this species will be detected again with great probability. Thus, studying the material of 2016–2017, the first author observed *T. titanica* in 7 of 50 chambers with bark of *Chosenia arbutifolia*, *Kalopanax septemlobus*, and *Populus maximowiczii* (i.e., in 14 % of cases), while processing the material collected in 2020, with this species being paid

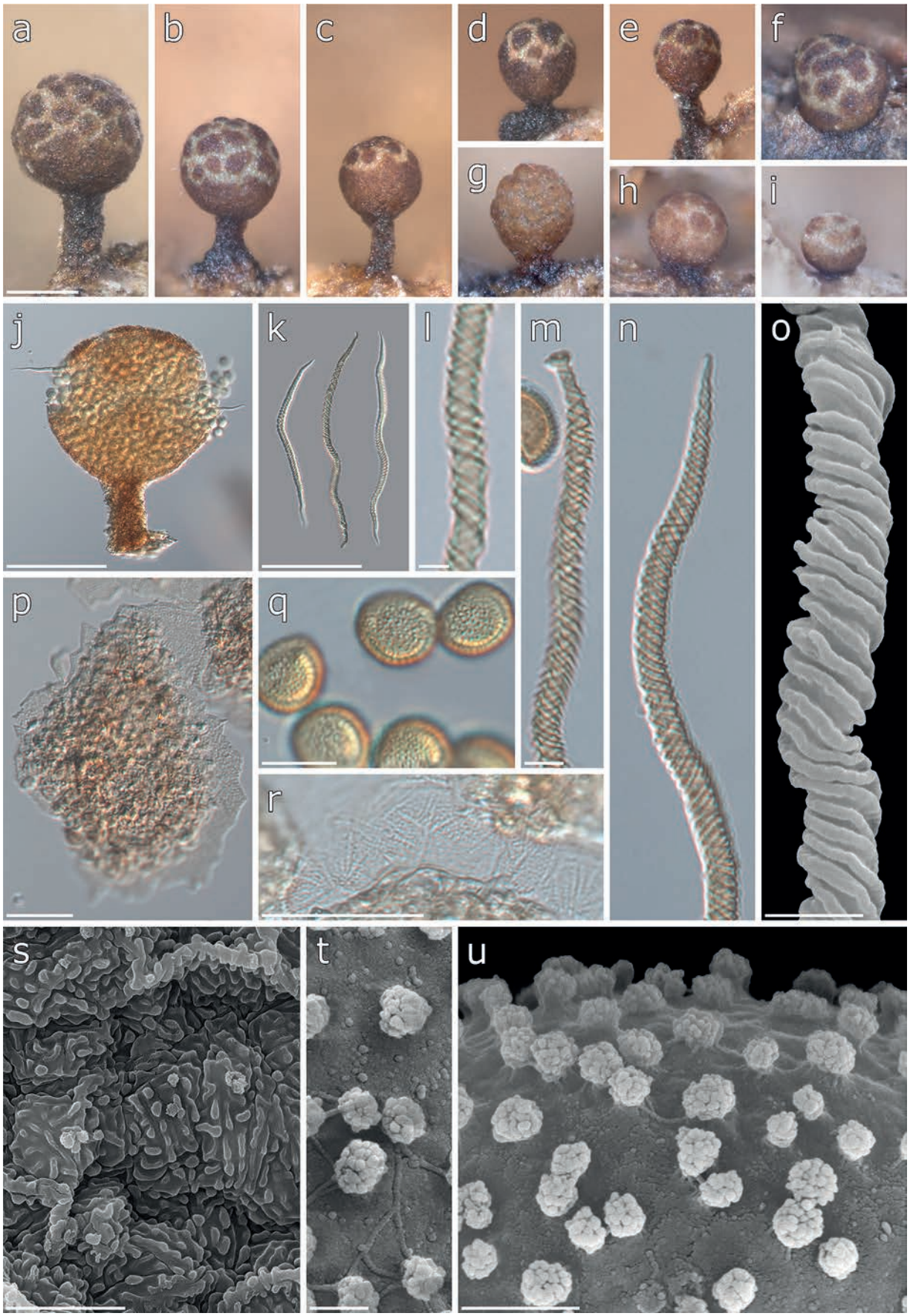

Figure 15 *Trichia titanica* **sp. nov.** (a–u): a–i – sporocarps (f – aberrant pulvinated sessile sporocarp), j – sporocarp under light microscope (LM), k, l – elaters (LM), m, n – tips of elaters (LM), o – elater under scanning electron microscope (SEM), p, r – warted peridium (LM), q – spores (LM), s – inner peridium (SEM), t, u – spore ornamentation (SEM). a – from MYX 21475, b – from MYX 21437, c, d, e, j, k, m, n, o, p, q, s, t, u – from MYX 21439 (**holotype**), f – MYX 21458, g, r – from MYX 10041, h – from MYX 21444, i – from MYX 21449, l – from MYX 21447. Scale bars: a–j – 100 μm, k – 50 μm, p, r – 20 μm, q – 10 μm, m, n – 5 μm, l, o, s – 3 μm, u – 1 μm, t – 0.5 μm

close attention to, the first author detected it in 29 of 55 chambers with the same tree species (i.e., in 53 % of cases).

The closest species to *T. titanica* is *T. flavicoma*, which, however, has larger sporangia, according to the original description: its stalk alone is 250 μm high (Lister 1894), whereas stalks of *T. titanica* are 31–83 μm high avg., and even the total sporocarp height exceeds 250 μm very seldom. We compared *T. titanica* with specimens from the Russian Far East and Vietnam, which we had attributed to *T. flavicoma*, although they were smaller than those described by Lister (see above). These two species were shown to have many features in common: general habit of sporangia, smooth elaters with the same length of tips, and practically identical ornamentation of the inner peridium and spores under SEM. Nevertheless, there are also significant differences.

First, the most pronounced difference lies in the length of elaters. Elaters of *T. titanica* are short, (35–) 70–120 (–165) μm long, not very elastic (Fig. 15k), while those of *T. flavicoma* are long, (150–) 200–370 (–540) μm, more elastic and often twisted (Figs 13g, h). For instance, the entire capillitium was represented by only one or few long elaters tangled in a ball even in the smallest *T. flavicoma* sporangium we studied, which was similar in size to *T. titanica* (Fig. 13g). Moreover, sporangia of *T. flavicoma* are 1.5 times avg. larger than sporangia of *T.titanica*, elaters are 2.9 times avg. longer. Also, species with even much bigger sporangia than *T. flavicoma* may be characterized by shorter elaters (see Fig. S9). Thus, these observations show that elater length does not directly depend on the sporotheca size. In addition to the elater length, density of spiral bands also differs: *T. titanica* is characterized by (14–) 15–18 (–19) spiral bands per 20 μm segment, while *T. flavicoma* has ca. (6–) 8–14 bands per the same segment. Second, these species are unlike in ecology: *T. titanica* is an exclusively corticolous species, whereas *T. flavicoma* is more often associated with litter (as Lister noted in the original description). Third, *T. flavicoma* has spores bright yellow in mass (*T. titanica* has dull yellow ones), and therefore dehiscence lines on the peridium look brighter and more contrasting (Fig. 13a–f). Fourth, the spore size is different: specimens of *T. flavicoma* from the Russian Far East have larger spores than those of *T. titanica* (10.7–12.2 μm vs 9.8–11.2 μm), while, in contrast, specimen of *T. flavicoma* from Vietnam has smaller spores (9.1–10.0 μm vs 9.8–11.2 μm). Certainly, this feature cannot be considered absolutely reliable, since the spore diameter range of *T. flavicoma* overlaps with that of *T. titanica*. Molecular data also indicate the similarity of these species (Fig. 1), although partial sequences of nrSSU, mtSSU, and EF1α from *T. flavicoma* and *T. titanica* have specific substitutions which allow to distinguish one species from the other.

Trichia rapa Bortnikov & Gmoshinskiy
sp. nov. Figs 16, S6–S11

MycoBank: 848468.

Etymology: from the Latin *rapum* – "turnip, tuber" due to the tuber-like shape of sporangia.

Description. Sporocarps sessile or stalked sporangia, solitary or in dense groups of 2–3, sometimes slightly deformed because of mutual compression, 600–800 μm high. Stalk, if present, finely furrowed, short, 100–190 μm, 16–24 % TH, expanding at the top, black, filled with refuse matter. Sporotheca globose to slightly flattened, 500–650 μm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with thin straight striae, sinuous veins, and "porous fields" – clusters of very small invaginations or perforations (visible by both LM and SEM). Outer layer made of granular matter brown to drab or black; deposits fairly thin and rather evenly distributed, so dehiscence lines very blurry and confluent. Dehiscence more or less irregular. Capillitium consisting of yellow elaters, pale yellow in TL, quite short, usually simple, (68–) 93–207 (–281) μm (n = 34) long and (3.2–) 3.5–4.4 (–4.9) μm (n = 18) wide. Capillitial threads decorated with left-handed spiral bands, very densely arranged, ca. 11–15 (–17) turns per 20 μm. Under SEM, spiral bands not smooth, with slight longitudinal striation. Secondary ornamentation of longitudinal or more often somewhat oblique anastomosing striae, so spaces between turns sometimes look venous or fibrous. Tips of elaters medium, ca. 35 (20–46) μm long, gradually tapering to a blunted end. Spores light yellow in mass, pale yellow in TL, sometimes subglobose, but usually of slightly irregular shape, warted, (10.5–) 11.5–12.9 (–14.2) μm diam. (Mean = 12.16; SD = 0.70; n = 66). Under SEM, spore ornamentation close to baculate: heads not assembled into large caputs, which is typical of pila, and bacula remain straight or even tapering upwards. High-definition SEM reveals secondary ornamentation of abundant tiny verrucae and thickenings, together forming a scabrous pattern. Plasmodium not observed.

Habitat: grass litter at the old overgrowing burnt area (in moist chamber).

Distribution: Russia: European part (Republic of Mordovia).

Holotype: RUSSIA, Republic of Mordovia, Smolny National Park, old overgrowing burnt area on the edge of a pine forest, 54.76114°N 45.44555°E, 16.11.2019, leg. A.I. Sheremeteva, I.M. Golikova, on grass litter (in moist chamber) (MYX 14188).

Notes: this specimen was originally identified as *T. flavicoma* using available identification keys (e.g., Poulain et al. 2011). However, it differs from *T. flavicoma* in larger sporocarps, indistinct peridium plates, and a very short stalk, which is often absent. In addition, specimen of *T. rapa* is different from our *T. flavicoma* specimens in close to baculate spore ornamentation (though visible only under SEM) and rough elaters which have striae both on spiral bands and between them.

Sessile sporangia of *T. rapa* bear a certain resemblance to those of *T. sordida*, a nivicolous species, because of the external habit (Johannesen 1984). However, in addition to the most important difference – the on in ecology – between these species, *T. sordida* has clearly pilate, not baculate spore ornamentation, elaters with smooth spiral bands, no distinct secondary ornamentation, and inner peridium decorated with a mosaic pattern of striae intersecting at different angles, without perforations or curved striae. In addition, spores of *T. sordida* are larger than those of *T. rapa* (14–15 μm vs 11.5–13 μm).

Trichia rapa differs from another sessile species, *T. contorta*, in having almost globular sporotheca, never elongated up to a plasmodiocarp. There are also differences in the ornamentation of spores and capillitial threads – unlike *T. contorta*, *T. rapa* has baculate spores and smoother spiral bands (Rammeloo 1974b, Ronikier et al. 2020). In addition, elater tips never branched and no swellings on the elaters have been observed. The similarity in ornamentation of inner peridium is certainly worth noting. The pattern of smooth lines and curved swellings like sinuous veins together with the ornamentation of invaginations is unique within the *Trichia botrytis* complex, but typical of *T. contorta*. The differences between two species are confirmed by molecular methods: *T. contorta* and *T. rapa* are clearly distant from each other on the phylogenetic tree.

Interestingly, according to the phylogenetic analyses (Fig. 1), the taxon most closely related to *T. rapa* is the undescribed OTU (operational taxonomic unit), whose nrSSU sequences were obtained during the analysis of RNA viral and eukaryotic host communities from the California annual grassland soil (Starr et al. 2019).

Trichia munda (Lister) Meyl. Figs 17, S6–S11

Description. Sporocarps stalked sporangia, scattered, 960–1800 μm high. Stalk rough, finely tuberous, rather long, 670–960 μm high, 52–72 % TH, 55–200 μm wide, orange-brown to almost black near the base, filled with refuse matter. Sporotheca single, obovate, 210–550 μm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, smooth, decorated with thin straight or sinuous striae, intersecting at different angles, usually not forming a complete net (Fig. 17m), but sometimes connecting (Fig.17l); pattern on the whole looks somewhat chaotic.

Outer layer made of granular matter, dark orange-brown to dark brown; very contrasting due to the distinct margins of peridium plates and lines 20–50 μm wide between them, through which bright spore mass can be seen. Dehiscence by preformed lines. Capillitium consisting of yellow to rusty yellow elaters, pale yellow in TL, short, usually simple, but sometimes singly branched, (70–) 100–270 (–480) μm

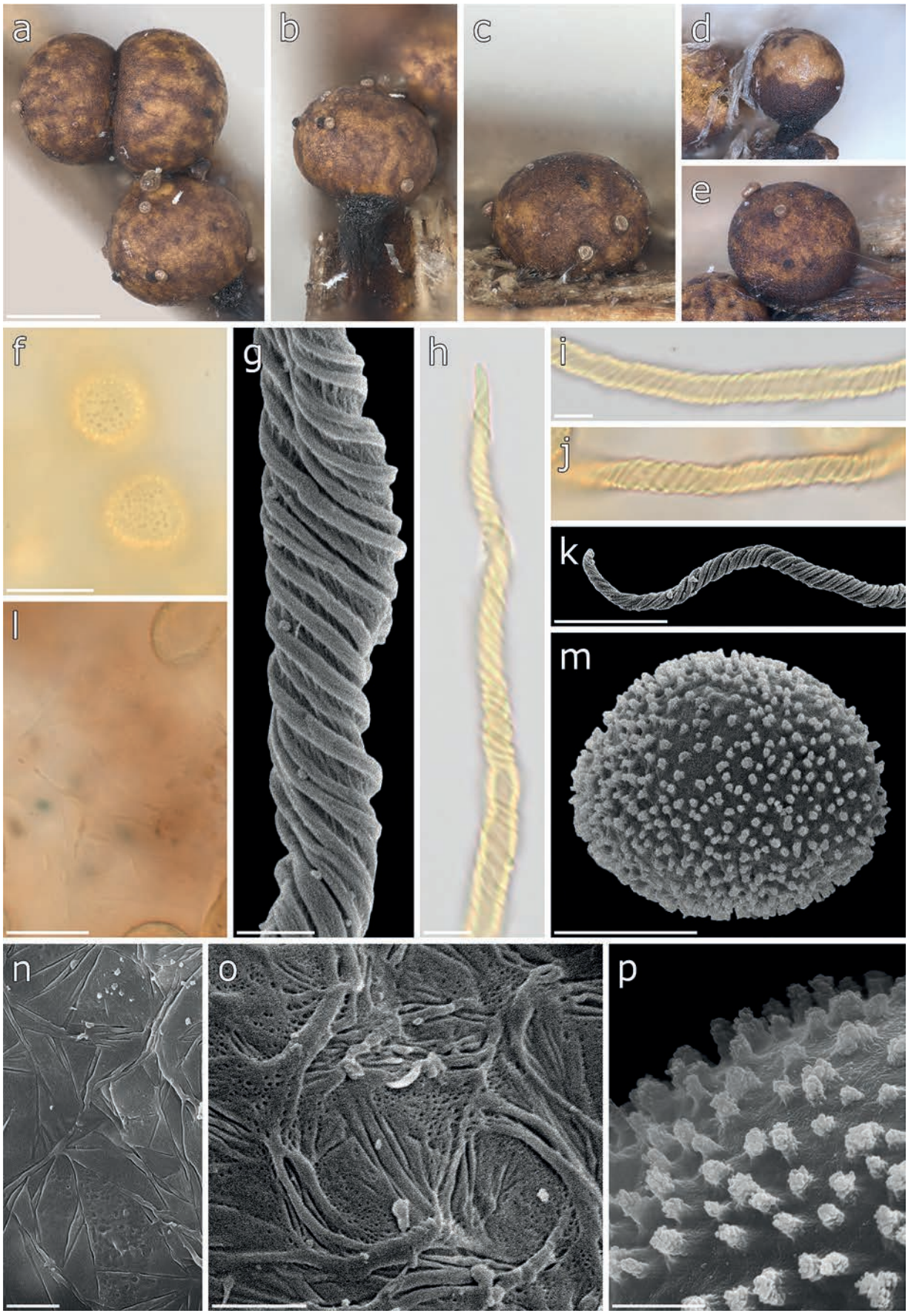

Figure 16 *Trichia rapa* **sp. nov.** (a–p): a–e – sporocarps, f – spores (LM), g – elater ornamentation (SEM), h–j – elaters (LM), k – tip of elater (SEM), l – peridium (LM), m – spore (SEM), n, o – inner peridium (SEM), p – spore ornamentation (SEM). a–p from MYX 14188 (**holotype**). Scale bars: a–e – 500 μm, f, k, l – 10 μm, h, i, j, m – 5 μm, n, o – 3 μm, g – 2 μm, p – 1 μm

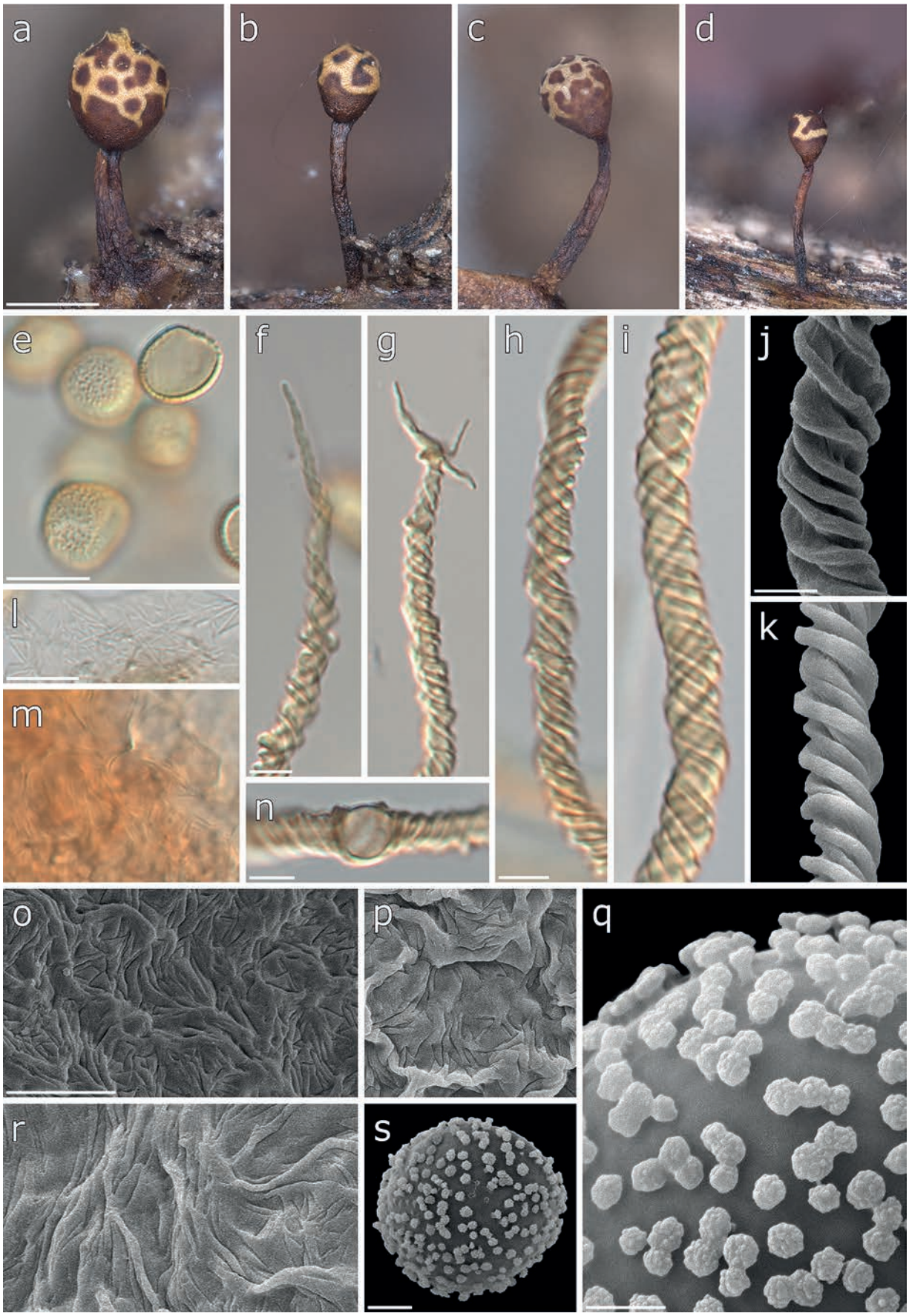

Figure 17 *Trichia munda* (Lister) Meyl. (a–q): a–d – sporocarps, e – spores (LM), f, g – tips of elaters (LM), h, i – elaters (LM), j, k – elaters (SEM), l, m – peridium (LM), n – cyst-like swelling in elater (LM), o, p, r – inner peridium (SEM), q, s – spore ornamentation (SEM). a, d, e, f, j, l, m, o, q, s – from LE 277815, b, i – from LE 277816, c, g, k, p – from LE 277828, h, n, r – from LE 277799. Scale bars: a–d – 500 μm, e, l, m – 10 μm, f, g, h, i, n, o, p, r – 5 μm, j, k, s – 2 μm, q – 1 μm

(n = 132) long and (3.1–) 3.7–4.7 (–5.1) μm (n = 70) wide. Elaters usually gradually tapering in one tip or abruptly tapered and branched into 2–3 very short thin tips (Fig. 17g). Unbranched tips medium, ca. 31 (21–48 μm) μm long. Capillitial threads decorated with left-handed spiral bands, densely arranged, with ca. 12–15 turns per 20 μm. Spirals of different height: some turns protruding for about 0.6–1 μm from the mean elater width, so that ornamentation looks rough and uneven. Under SEM, spiral bands smooth, without prominent secondary ornamentation. Spores bright yellow in mass, pale yellow in TL, sometimes subglobose, but usually of somewhat irregular shape, ornamented with scattered warts (8.2–) 9.2–10.6 (–12.5) μm diam. (Mean = 9.93; SD = 0.69; n = 337). Under SEM, spore ornamentation pilate: columnar structure usually stumpy, caputs wide, with grouped rounded heads; sometimes pila joined in groups of 2 or 3 by caputs. Secondary ornamentation absent. Plasmodium not observed.

Habitat: leaf litter of *Quercus robur*, pH 6.2–6.7 (in moist chambers).

Distribution: Russia: European part (Volgograd Region).

Studied specimens: see table S1.

Notes: *Trichia munda* is a taxon with a rather vague concept, which seems to be understood differently by different authors. Lister, describing *T. botrytis* var. *munda* in 1897, noted that the new species differs from the type variety by slightly smaller sporangia, elaters about 3.5 μm wide with medium-long tips, and preference for oak and hornbeam ground litter (less frequently dead wood). The main feature, however, for which the name munda was given, is very dense and neat arrangement of spiral bands on elaters (Lister 1897). In 1927, Meylan assigned species rank to the taxon, primarily due to the difference in the plasmodium color (white rather than black and purple as that of typical variety of *T. botrytis*). In the analytic table (key), he also noted that *T. munda* has elater tips 20–40 μm long and spores 9–12 μm and inhabits dead bark and rotten wood (Meylan 1927). Although long stalks greatly exceeding the diameter of sporotheca had not been mentioned in any way in the original description, this trait was noted by several authors (e.g., Ing 1999) and even suggested as a diagnostic feature of *T. munda* (Poulain et al. 2011).

The total size of sporangia also varies considerably among different publications: Yamamoto (2006) indicates the height of up to 0.3 mm, Liu et al. (2002b) – 0.58–0.71 mm, Stephenson (2021) – 0.6–1.5 mm. In his description, Ing (1999) states that sporangia are up to 1.5 mm high, but his drawing, according to a scale bar, shows the sporangium about 2 mm high. Such large sporangia (about 1.9 mm) are also depicted in Johannesen & Vetlesen (2020).

Substrates on which *Trichia munda* has been found include litter (e.g. Lister 1897), wood (e.g. Meylan 1927), and the bark of living trees (e.g.Härkönen et al. 2004). We believe that such broad spectrum actually represents not a great adaptability of the species, but a rather poorly developed species concept.

The specimens we attribute to *Trichia munda* were obtained in moist chambers on leaf litter of *Quercus robur*; they had elaters 3.1–5.1 μm wide with medium tips (21–48 μm) and spores averaging 9.2–10.6 μm diam., which more or less agrees with descriptions of Lister and Meylan (Lister 1897, Meylan 1927). At the same time, they had rather large sporangia (sometimes up to 1.8 mm high) and long stalks (52--72 % of the total height), as it is stated in other works (Ing 1999, Poulain et al. 2011, Johannesen & Vetlesen 2020). It is important to note that studied specimens had one obvious difference from the original description: spiral bands decorating elaters can hardly be described as neat and evenly spaced, which may be a result of aberrant development. We found cyst-like swellings in the elaters of studied specimens, especially in those of LE 277799 (Fig. 17n), which are very similar to the thickenings described as structures of a Cryptomycota representative (Yajima et al. 2013). Assuming this to be the case, development of a different organism within the elaters might have influenced their morphogenesis. This, however, requires a specific study.

Recently, syntype of *Trichia munda* (BM 2942) has been studied by Moreno et al. (2022). They state that the specimen was collected on the leaves of hornbeam in 1896 (rather than oak in our case), sporocarps are 1.0–1.5 mm high (compared to 0.96–1.80 mm in our case), stalk is 2–2.5 times the diameter of sporotheca (compared to 1.2–3.2 times, 2.3 times avg.), elaters are 3–5 μm wide (compared to 3.7–4.7 μm avg.), elater tips are up to 40 μm long (compared to 21–48 μm), and spores are 9–12 μm diam. (9.2–10.6 μm avg.). Moreover, SEM photos prove that elaters have no secondary ornamentation, which agrees with our observations, although elaters of our specimens bore slightly less neat spirals. The only noticeable difference is that SEM reveals that adjacent pila on the spore surface are sometimes joined by thin bridges at the top, a feature we state for our specimens of *T. ambigua* (see further) and *T. botrytis*, but not for *T. munda*, which sometimes has joined caputs, but never long thin bridges. Unfortunately, authors of the study do not provide photographs of the sporangium habit or description of the outer peridium, and there are no data on the elater length and ornamentation of the inner peridium.

Eventually, despite slight discrepancy with the original description and the study of syntype, we define our specimens exactly as *Trichia munda*. The next important and useful step would be to study fresh material collected in the type locality for morphological and genetic comparison.

Trichia armillata Bortnikov **sp. nov.** Figs 18, S6–S11

MycoBank: 848476.

Etymology: from the Latin *armilla* – "ring, hoop"; there are usually few peridium plates (often 3–5), and sometimes there is just one plate, so the circular dehiscence line girds sporotheca like a ring.

Description. Sporocarps stalked sporangia, scattered, (390–) 510–730 (–930) μm high. Stalk rough, finely tuberculous, rather long, 160–580 μm high, 36–69 % TH (46–60 % avg.), light-orange-brown to dark brown or blackish-brown, filled with refuse matter. Sporotheca single, subglobose to obovate, 180–380 μm wide. Peridium two-layered. Inner layer thin, membranous, hyaline. Decorated with short straight striae intersecting at different angles, constituting a mosaic pattern. Outer layer made of granular matter, yellow-brown to dark brown and blackish-brown. Dehiscence by preformed lines. Capillitium consisting of yellow elaters, pale yellow in TL, simple, (80–) 170–290 (–370) μm (n = 140) long and (2.6–) 3.3–4.1 (–4.6) μm (n = 92) wide. Tips of elaters short to medium, ca. 23 (14–36) μm long, gradually tapering. Capillitial threads decorated with left-handed spiral bands, arranged loosely, but quite evenly, with ca. (8–) 9–11 turns per 20 μm. Under SEM, spiral bands smooth, without secondary ornamentation. Spores dull yellow in mass, pale yellow in TM, usually subglobose, but sometimes of somewhat irregular shape, (8.2–) 9.2–10.8 (–13.3) μm diam. (Mean = 10.03; SD = 0.80; n = 529). Under SEM, spore ornamentation pilate: columnar structure stumpy, caputs wide, with grouped rounded heads. High-definition SEM reveals secondary ornamentation of minute verrucae, more or less evenly arranged, sometimes uniting in short lines (Fig. 18q). Plasmodium not observed.

Habitat: bark of living trees (*Abies holophylla* Maxim., *Quercus mongolica*) with median pH of 5.4 (in moist chambers).

Distribution: Russia: Far East (Primorye Territory).

Holotype: RUSSIA, Primorye Territory, Kedrovaya Pad Nature Reserve, dry mixed Ussurian forest dominated by *Abies holophylla* with an admixture of *Tilia amurensis* and *Quercus mongolica*, 43.119222°N 131.470250°E, 10.03.2018, leg. F.M. Bortnikov, on the bark of living *Abies holophylla* (in moist chamber), pH 5.47 (MYX 11050).

Other studied specimens (paratypes): see table S1.

Notes: initially most of the specimens assigned to this new species were identified as *Trichia munda* (some of them – as *T. botrytis*) because of relatively long stalks (sometimes up to 70 % TH), moderately long elater tips (14–36 μm), and spore diameter falling within the 9–12 μm range. Nevertheless, there are two features of *T. armillata*, disagreeing with the original description of *T. munda* (Lister 1897).

It is rather difficult to call the peridium of studied specimens mottled, the feature Lister indicated for *Trichia munda*, since it usually consists of only a few large plates (typically 3–5, rarely more, sometimes only one). Moreover, the studied spe-

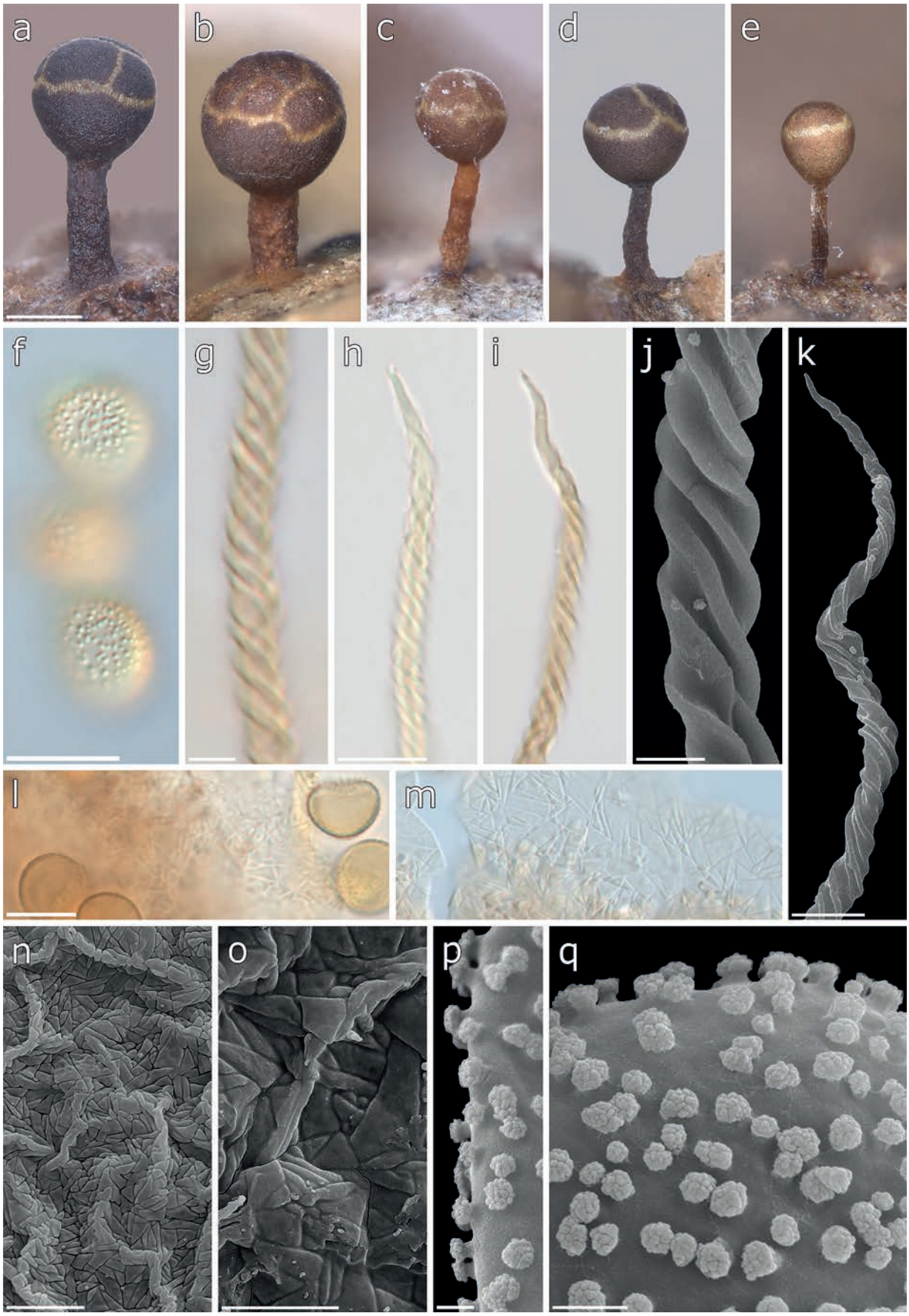

Figure 18 *Trichia armillata* **sp. nov.** (a–q): a–e – sporocarps, f – spores (LM), h, i – tips of elaters (LM), j, k – elaters (SEM), l, m – inner peridium (LM), n, o – inner peridium (SEM), p, q – spore ornamentation (SEM). a, o – from MYX 11027, b, f – from MYX 21426, c, i, m – from MYX 11173, d, h, l – from MYX 11050 (**holotype**), e, g, j, p – from LE F-348755, k – from MYX 11083, n, q – from LE 308159. Scale bars: a–e – 200 μm, f, h, i, l, m – 10 μm, k, n, o – 5 μm, g – 3 μm, j – 2 μm, p, q – 1 μm

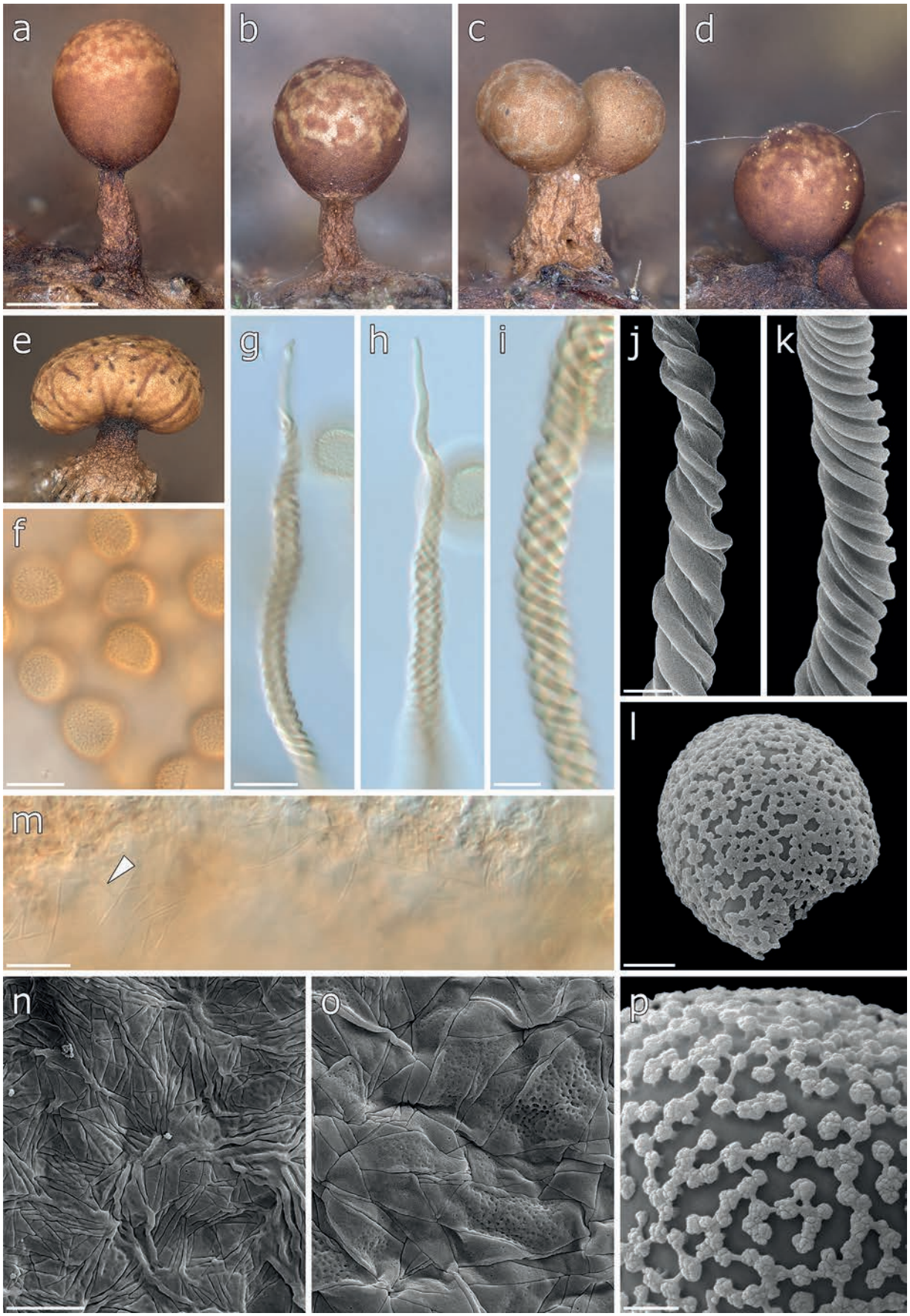

Figure 19 *Trichia ambigua* Schirmer, L.G. Krieglst. & Flatau (a–p): a–e – sporocarps (e – aberrant form), f – spores (LM), g–i – elaters (LM), j, k – elaters (SEM), l – spore (SEM), m – peridium (LM), n, o – inner peridium (SEM), p – spore ornamentation (SEM). a, g, n, p – from MYX 13072, b, m – from MYX 13106, c – from MYX 13086, d, f, k, o – from MYX 13071, e – from MYX 18828, h, i – from MYX 13113, j, l – from MYX 13116. Scale bars: a–e – 500 μm, f, g, h, m – 10 μm, i, n, o – 5 μm, j, k, l – 2 μm, p – 1 μm

cimens inhabited the bark of living trees and have never been found on other substrates, such as those stated in the original description by Lister, namely dead leaves of oak, hornbeam, etc., rarely wood. Although in two cases specimens were collected from litter, sporangia were still located on small pieces of fallen bark. In addition, studied specimens had smaller sporangia than usually reported for *T. munda* (Ing 1999, Stephenson 2021).

Phylogenetic analysis shows that *Trichia armillata* is a sister taxon to the specimens we assigned to *T. munda*; however, *T. armillata* is characterized by specific substitutions and forms a monophyletic clade with maximum supports (Fig. 1).

Trichia ambigua Schirmer, L.G. Krieglst. & Flatau Figs 19, S6–S11

Description. Sporocarps stalked or rarely almost sessile sporangia, scattered, 790–1560 μm high. Stalk furrowed, rather short, (45–) 155–720 μm high, 20–46 % TH, usually light brown, but rarely to almost black, more or less evenly colored, filled with refuse matter. Sporotheca single or sometimes in groups of 2–4 on a common stalk, obovate, 530–760 μm wide. Peridium two-layered. Inner layer thin, membranous, hyaline, decorated with straight or slightly curved lines and striae of various size, intersecting at different angles. Secondary ornamentation of groups of invaginations between striae sometimes present (Fig. 19m, n, o). Outer layer made of granular matter, light yellowish-brown to reddish-dark-brown, looking like numerous spots. Due to the gradual thinning of deposits, dehiscence lines smooth and blurry, peridium plates often of irregular shape. Dehiscence by preformed lines or almost irregular when peridium plates not pronounced. Capillitium consisting of yellow or brown-yellow elaters, pale yellow in TL, always simple, (140–) 260–470 (–670) μm (n = 215) long and (3.7–) 4.1–4.9 (–5.7) μm (n = 115) wide, sometimes intertwisted into double spirals. Tips of elaters medium, ca. 38 (32–48) μm long, gradually tapering. Capillitial threads decorated with left-handed spiral bands arranged evenly and quite densely, ca. 11–14 (–15) turns per 20 μm. Under SEM, smooth, without any secondary ornamentation between the spirals. Spores yellow in mass, pale yellow in TL, usually subglobose, but sometimes of slightly irregular shape, minutely and densely warted, (10.1–) 10.7–11.8 (–13.0) μm diam. (Mean = 12.26; SD = 0.54; n = 482). Under SEM, spore ornamentation pilate: pila often arranged in short lines or clusters joined by bridges between caputs. Secondary ornamentation absent. Plasmodium not observed.

Habitat: rotten wood (in the field).

Distribution: Russia: European part (Moscow and Tver Regions), North Caucasus (Karachay-Cherkess Republic); Germany (Bavaria).

Studied specimens: see table S1.

Notes: the studied specimens of *Trichia ambigua* from the European part of Russia fit the original description very well and undoubtedly belong to this species. In one of the examined specimens (MYX 18828) there are, in addition to sporangia with typical peridium morphology but very short stalks, several aberrant fruiting bodies completely unlike *T. ambigua* and many sporangia are characterized monochrome black stalks (Fig. 19e). However, both micromorphological and molecular comparisons reliably confirmed its conspecificity with other specimens.

Trichia ambigua was described during the revision of European specimens previously identified as *T. subfusca*, which, in turn, was described from North America (Rex 1890, Schirmer et al. 2015). We agree with the authors of the study (Schirmer et al. 2015) that *T. subfusca* is an extremely often misinterpreted species and is in fact very rare. Among the specimens used in this work, at least 7 of them were previously identified as *T. subfusca* because of the low-contrast peridium plates, but after detailed examination they turned out to be anything but *T. subfusca*, including species new to science (*Trichia acetocorticola* **sp. nov.**, *T. ambigua*, *T. botrytis* s. s., *T. musicola* **sp. nov.**, *T. nubila* **sp. nov.**). It is worth noting that phylogenetic analyses show that the only nrSSU sequence of *T. subfusca* (KT358726) available in GenBank is from Germany and also falls into the *T. ambigua* clade with the highest support values (Fig. 1).

Unfortunately, among the specimens we studied in the LE and MYX fungaria, there was not a single one that could be reliably attributed to the species *T. subfusca*. Therefore, we consider it important to focus once again on the most significant differences between these two species, using the information given in the original description of *T. subfusca* and our data on *T. ambigua*. The most reliable differences involve not the features of peridium, but of capillitium: elaters of *T. subfusca* are thinner than those of *T. ambigua* (3.5–4 μm vs 4.1–4.9 μm) and elater tips are dramatically shorter (10–12 μm vs 32–48 μm) (Rex 1890). We could not compare the elater length of these species due to the absence of information in the original description by Rex. Because of the absence of specimens reliably identified as *T. subfusca* at our disposal, it is also not possible to speak about its phylogenetic position yet.

DISCUSSION

Taxonomic significance of traits for the delimitation of taxa at the species level and limits of their variability were evaluated based on the literature and own observation.

Plasmodium

Previously, some authors used plasmodium color (white, blackish-purple, chocolate, etc.) to support the delimitation of species in the *Trichia botrytis* complex (e.g., Meylan 1927). However, this trait, apart from the obvious problem of subjective color perception, is simply difficult to observe and requires special study of the sporulation process. Plasmodia cannot always be examined even in moist chambers (e.g., due to the small size or ephemerality) and especially under field conditions during routine collecting. Therefore, it can be used only as an additional feature, but not a diagnostic one.

Sporocarps

All the morphotypes studied had sporangia, sometimes on short stalks, but never exclusively sessile or plasmodiocarps.

Most species form fruiting bodies in more or less scattered groups, although there are those that always form individual sporangia (e.g., *Trichia titanica* or *T. munda*), or, contrariwise, very densely crowded colonies (*Trichia acetocorticola* var. *aggregata*).

The size of sporangia (see Fig. S7) among the studied species can be useful and truly diagnostic perhaps only for minute species like *Trichia armillata*, *T. flavicoma*, and *T. titanica* (the latter is the smallest species of *Trichia* described so far). *Trichia nubila* and *T. rapa* also have rather small sporangia due to the very short stalks, while their sporothecae are comparable in size to those of other species. Usually, total height of other species varies by about 1–1.5 mm.

The sporocarp color within the *Trichia botrytis* complex is based on the proportion of the following shades: yellow, brown, red, black, and gray. The first one is dictated by the color of the spore mass showing through the translucent inner peridium, while the others are mainly determined by the structure and thickness of deposits of the outer peridium.

The accurate assessment of the taxonomic significance of all these traits is complicated by the fact that they are usually expressed rather uniformly within a single colony, and the study of large number of specimens is, firstly, quite laborious due to the need for reliable confirmation of conspecificity by different methods, and secondly, sometimes simply impossible due to the extreme rarity of particular species. For example, about 60 colonies of *Trichia titanica* were studied, and the indicated limits of variability can be

regarded as sufficiently credible, which cannot be claimed for the species represented so far by a single specimen (*T. acetocorticola* var. *aggregata*, *T. gradalia*, *T. nubila*, and *T. rapa*).

The practical use of this set of traits sometimes causes difficulties, mainly when dealing with specimens found in the field, because it is quite common to find old, severely damaged colonies preserved from autumn, or fresh colonies, but already dehisced. In both cases, microscopic features are usually well preserved, while macroscopic features are lost due to the peridium rupture.

Thus, the habit of fruiting bodies is important, but these features should be used with caution and always only in combination with other, microscopic, ones.

Hypothallus and stalk

Hypothallus of most species of the *Trichia botrytis* complex is either practically absent or very thin and translucent. However, there is an exception: hypothallus of *T. gradalia* is sometimes well defined, disc-shaped, contrasting to the substrate color. The internal structure of the stalk is also quite constant in all species of the complex: it is filled with refuse matter, and stalks with spore-like cells (as, for example, in case of *Trichia decipiens* (Pers.) T. Macbr. ≡ *Hemitrichia decipiens* (Pers.) García-Cunch. Zamora & Lado) have not been observed. The only significant features may be the color of the stalk (light or dark, evenly colored or gradient), structure of its surface (tuberculous, furrowed, deeply wrinkled), and the place of its contact with sporotheca (for example, large wrinkles from the stalk can extend to the base of sporotheca, which is characteristic only for *T. gradalia*).

Outer peridium

The outer peridium of all studied species is the same: it consists of granular material deposits. For this reason, SEM photographs of the outer peridium surface, including photographs of the entire sporangium (e.g., Novozhilov et al. 2009, fig. 14, Zhang & Li 2016, fig. 3c) are not of great importance if there is a regular macrophotograph in reflected light.

We consider the degree of gradual thinning of deposits near the dehiscence lines, where the inner peridium is exposed, to be an additional or even diagnostic feature. In some cases (e.g., *T. musicola* and especially *T. rapa*), the deposits fade smoothly and the dehiscence lines are blurred (Figs 12, 16), and in other cases (*T. munda*, *T. papillata*), they terminate abruptly so that the dehiscence lines look very contrasting (Figs 11, 17).

Another important diagnostic trait is the number of peridium plates, which may vary from 2–3 per visible part of sporotheca (*Trichia armillata*) to more than 10 (*T. erecta*). This trait, however, is difficult to consider universal for two reasons: first, the margins of peridium plates are sometimes so vague, that it is almost impossible to understand what is supposed to be considered a separate plate (e.g., *T. musicola*, *T. gradalia*, or *T. rapa*), and second, in some cases (e.g., *T. ambigua* or *T. pinicola*), apart from large peridium plates, there are also very small ones in the gaps between them, that can almost certainly lead to different interpretation of their number by different researchers. If this trait can still be used, it is necessary to operate averaged values, since intraspecific variability may be quite high.

The outer peridium of *Trichia papillata* is unique: it consists of polygonal plates with very contrasting margins and a papilla-like thickening in the center of every plate (Adamonytė 2003, Liu et al. 2007, Gmoshinskiy et al. 2019, Fig. 11). In case of *T. botrytis* var. *cerifera*, the outer surface of peridium is covered with nearly rounded wax-like deposits, and that also deserves attention in the future studies.

Inner peridium

In the majority of publications, the inner peridium in the *Trichia botrytis* complex is characterized only as a translucent membranous layer visible between peridium plates (Martin & Alexopoulos 1969, Ing 1999, Poulain et al. 2011). We are aware of only one detailed study of the inner peridium in the whole family Trichiaceae using SEM; it was conducted by Rammeloo (1974a), who included *T. botrytis* and *T. erecta* among other species. He also noted that differences in peridium ornamentation can be used to elucidate relationships within the genus *Trichia*.

We support this point of view and believe that the type of inner peridium ornamentation is a very important feature that should be paid close attention to. Among studied species of the *Trichia botrytis* complex we distinguish the following main types of ornamentation:

1A. Straight dashes and lines, intersecting at different angles and uniting in a mosaic pattern. Sometimes, but not always, numerous invaginations are located in the center of each fragment of such mosaic (*T. acetocorticola*, *T. ambigua*, *T. armillata*, *T. botrytis*, *T. munda*, *T. nubila*, *T. pinicola*).

1B. Straight lines combined with curved lines and swollen veins. Fragments of mosaic are almost always dotted with numerous small invaginations or perforations (*T. rapa*).

1C. Short and medium-size dashes, forming a mosaic pattern and usually not intersecting with each other (*T. botrytis*, *T. munda*, *T. taeniifila*).

1D. Numerous invaginations in the form of dots or very short dashes arranged in a rather chaotic order, very similar to those found in type 1A in the center of mosaic fragments, but lines and mosaic pattern are absent (*T. gradalia*).

2. Numerous verrucae, sometimes combined with lines forming a large mosaic pattern (*T. flavicoma*, *T. musicola*, *T. papillata*, *T. titanica*).

3. Numerous small dashes and tubercles, so that the whole pattern may resemble fine reptile scales (*T. erecta*).

These types of ornamentation are particularly well discernible under SEM, but are generally distinguishable even with a light microscope and oil immersion. Therefore, undoubtedly, this feature can be used as one of the important diagnostic ones. At the same time, it should be pointed out that, according to our observations, ornamentation of even one fruiting body may slightly vary: we found two similar types of ornamentation, 1A and 1C, simultaneously present in one sporangium in *Trichia botrytis* and *T. munda*. Also, there are specimens of *T. pinicola* both with and without invaginations in polygonal areas.

Additionally, in some cases, examination of the peridium under SEM at low magnification may cause an impression of reticulate ornamentation (see Fig. 5m), but in fact it is not an actual ornamentation, but an artifact arising from deep indentations left by spores during the development of sporocarp.

Capillitium

Before proceeding to the discussion of quantitative features, we should indicate that we are unaware of any methodological studies on the effect of the medium used (lactophenol, KOH, lactic acid, etc.) on the measurement results, such as changes in spore diameter, capillitium size and shape, and other morphological traits of myxomycetes.

All features of the capillitium (perhaps except for the color) are important diagnostic traits in case of the *Trichia botrytis* complex.

The elater width of the studied species of *Trichia* varied from 2.6 to 8.6 μm (see Fig. S8). This trait is quite consistent within species and sometimes allows to distinguish closely related ones, for example, *T. nubila*, *T. acetocorticola*, and *T. acetocorticola* var. *aggregata* with wide elaters from *T. pinicola* with thinner elaters, although these species are very close by other features and genetically related. The elater width is given in most descriptions published with an accuracy of 0.5–1 μm, though we believe that it should be stated with an accuracy of up to 0.1 μm.

The elater length is a feature that has not been previously studied for species of the *Trichia botrytis* complex (and *Trichia* in general) and is mentioned only in single publications for *T. botrytis* (Vasyagina et al. 1977), *T. macrospora* (Zhang & Li 2016), and *T. nivicola* (Kuhnt 2019). According to our observations, in most cases, the elater length is a fairly stable trait and can also help to distinguish closely related species; for example, *T. flavicoma* from *T. titanica*, or *T. pinicola* from *T. nubila*, *T. acetocorticola*, and *T. acetocorticola* var. *aggregata* (see Fig. S9). It is important to remember that sometimes aberrant elaters, clearly differing in length or shape from normal ones, can be formed.

The length of elater tips is another diagnostic feature (for example, in the aforementioned complex *nubila–acetocorticola–acetocorticola* var. *aggregata–pinicola*, *T. nubila* can be easily distinguished by its short tips). In many publications, however, the tip length is not precisely indicated, and the expression "endings short/medium/long" may be perceived very subjectively. In fact, most species have a very subtle, but clearly visible subterminal swelling prior to the tip, and therefore a fairly accurate measurement of the tip length is not a problem (see the explanation in the Material and Methods section and S4). The exceptions are *T. titanica*, *T. rapa*, *T. flavicoma*, and *T. munda*, whose elaters thin very gradually, but it is possible to measure even their tips with a small error. Among the studied species, the average length of tips varied from 7 μm (*T. erecta*) to 84 μm (*T. botrytis*) (Fig. S10).

The ornamentation of elaters is truly the most noticeable diagnostic feature of the capillitium. All species of the *Trichia botrytis* complex have elaters decorated with conspicuous spiral bands. Our observations show that almost all studied species (except for some specimens of *T. taeniifila*) are characterized by left-handed spirals, which is typical of most species of the genus *Trichia*. The direction of spirals is a trait that is very often overlooked. For example, in Neubert et al. (1993), illustrations of different species of *Trichia* often include both left- and right-handed spirals even for the same specimen, suggesting that drawings were made inattentively using different focal planes. The feature, however, is considered consistent and valuable, as shown earlier by other authors: for instance, one of the most important differences between *Hemitrichia montanoides* Mar. Mey. & Poulain and *H. montana* (Morgan) T. Macbr. is right-handed spirals on elaters (Poulain & Meyer 2007). At the same time, our data show that specimens of *T. taeniifila* with left-handed spirals and *T. taeniifila* with right-handed spirals have zero mismatches in nrSSU, mtSSU, and EF1α sequences, which brings up the question of the nature of spiral band direction and the potential use of this feature as a diagnostic one.

We classify ornamentation of spiral bands and space between them into seven different types:

1) spirals smooth; little or no secondary ornamentation (*T. ambigua*, *T. armillata*, *T. flavicoma*, *T. gradalia*, *T. munda*, and *T. titanica*). In some cases, there may be a false impression of a slight longitudinal striation between spirals (see Figs 13i–j, 17j), which we regard as an artifact caused by excessive drying of the elater;

2) spirals slightly longitudinally split, somewhat fibrous on the surface; little or no secondary ornamentation (*T. botrytis*);

3) spirals almost smooth; secondary ornamentation fine-fibrous (*T. rapa*);

4) spirals smooth or ornamented with spines; secondary ornamentation large-fibrous (*T. erecta*);

5) spirals fine-warted; secondary ornamentation almost absent (*T. musicola*, *T. papillata*);

6) spirals almost smooth or longitudinally furrowed; secondary ornamentation mesh-like or reticulate (*T. taeniifila*);

7) spirals rough, split, or reticulate; secondary ornamentation of longitudinal plications and small reticulate areas (*T. nubila*, *T. acetocorticola*, and *T. pinicola*).

These types of ornamentation are very well-visible under SEM, but in most cases can also be observed using oil immersion (see for example Figs 2h, 4g, 6h, 8i).

Finally, another feature, the density of spiral bands, can also be used as an additional (and sometimes possibly diagnostic) feature. We measured the absolute density of spirals (without correction for elater width) on a 20 μm segment and found that the lowest density was observed in *Trichia taeniifila*: 2–6 (up to 7) turns per 20 μm, and the highest was in *T. titanica*: 14–19 turns per 20 μm. Curiously, *T. flavicoma* (species very similar to *T. titanica*) is characterized by an average density of 8–14 spirals per 20 μm. Thus, although there are no data on this feature in the literature, we find it at least of interest and worthy of attention.

Spores

The shape of spores and their ornamentation in TL is not an important feature in this species complex, since all spores are almost globose or more often of somewhat irregular shape and simply warted. The spore wall is not quite evenly thickened in the case of most (or all) species, but this feature is not really conspicuous and can be overlooked or misinterpreted.

We tried to estimate the density of warts on epispore by manually counting their number in the spore central 3×3 μm region in a sample of 5 spores per species (Fig. 20). Indeed, the density of warts differs between species and ranges from 1.7–1.8 pc/μm^2 (*Trichia munda*, *T. rapa*, *T. musicola*, and *T. erecta*) to 3.29 pc/μm^2 (*T. ambigua*). However, it is difficult to use this feature due to some variability and possible subjectivity of the calculations. Moreover, this feature is rather

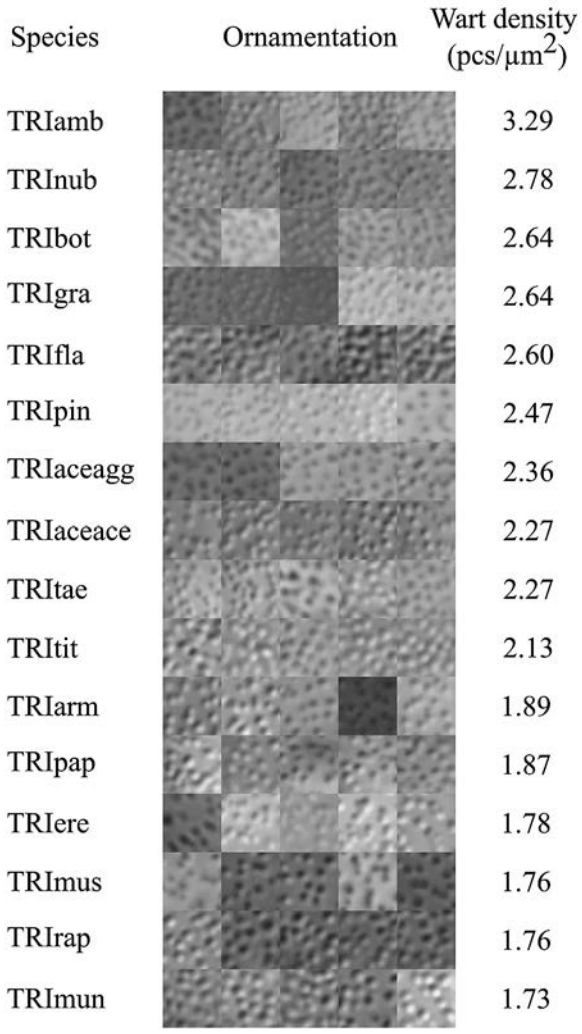

Figure 20 Density of warts on spores of different *Trichia* species

laborious to estimate and seems to be of low value, since it helps to distinguish the species, that can be easily told apart using other morphological features.

The spore size varies within a fairly wide range for myxomycetes: mean values among the studied morphotypes vary from 8.3 μm (*Trichia gradalia*) to 13.4 μm (*T. erecta*) with the minimum value of 7.68 μm and the maximum one of 14.55 μm (Fig. S11). However, the spore size is fairly stable within every species: the standard deviation averages 0.62 μm: from 0.34 μm (*T. gradalia*) to 1.01 μm (*T. flavicoma*). Therefore, this feature is valuable and diagnostic, provided it is measured accurately, uniformly, and in a sample of at least 20–30 (preferably 50) spores (see also Material and Methods). Also, one should keep in mind that in the case of incomplete sporocarp development the spore size can be significantly larger than the average, and such spores should be omitted from the measurements.

Another taxonomically important feature is the spore ornamentation under SEM. We have shown that species of *Trichia botrytis* complex normally have spores with pilate ornamentation, and only *T. erecta* and *T. rapa* have baculate ornamentation, not even quite typical in the latter case. Pilate ornamentation is variable: pila may be separate or connected with each other by thin bridges at the top of caputs, sometimes even by caputs themselves (for example, *T. ambigua*, see Fig. 19l). Caputs can also be different: either with coarse, widely spaced heads (Fig. 12r), with tightly packed or rounded heads (Fig. 11m).

In several cases, under SEM we observed secondary ornamentation of small verrucae, sometimes uniting in rows (Figs 13r, 15u, 18q), and of fibrous structures extending from the bases of pila (Fig. 12r). Despite the obvious taxonomic importance of this feature (e.g., *Trichia flavicoma* and *T. titanica*, two closely related species, that have almost identical secondary ornamentation untypical of other morphotypes), we believe that it should be considered diagnostic with great caution and preferably used only as an additional one. For example, secondary ornamentation was detected for some specimens of *T. erecta* and not for others (Fig. 2p, q), although they were identical in other features, including nrSSU sequences. According to our observations, secondary ornamentation is often visible only at the very high magnification under SEM (see *T. nubila*) and can easily be missed if microphotographs are of insufficient quality.

Ecology

Our data show that all (or almost all) species of the *Trichia botrytis* complex have stable ecological preferences even in different geographic regions. Thus, three ecological groups were identified:

Lignicolous species, predominantly found on rotten wood: *T. ambigua*, *T. botrytis*, *T. erecta*, *T. gradalia*, *T. nubila*, and *T. taeniifila*.

Foliicolous species, primarily occurring on ground or, less often, aerial leaf litter: *T. flavicoma*, *T. musicola*, *T. papillata*, and *T. rapa*. It is worth noting that *T. papillata* was originally described as coprophilous (Adamonytė 2003), but the specimen we studied was collected from ground leaf litter. *Trichia musicola* was found on banana leaf litter with pH about 7.3–8.3. In Russia, *T. flavicoma* was found on litter as well as on the bark of juniper, which was, however, a creeping shrub and its branches, covered by thin soft bark, were in direct contact with ground litter. In Vietnam, however, the only specimen of *T. flavicoma* was obtained from moist chambers with the bark of an undetermined deciduous living tree. Therefore, substrate preferences of these species require further study.

Corticolous species, observed only on the bark of living trees and shrubs so far: *T. acetocorticola*, *T. acetocorticola* var. *aggregata*, *T. armillata*, *T. pinicola*, and *T. titanica*. Moreover, all these species are confined to the bark of trees with a certain pH range: *T. acetocorticola*, *T. acetocorticola* var. *aggregata*, and *T. pinicola* were found on the bark of *Pinus koraiensis*, *P. massoniana*, and *Betula schmidtii* with median pH 4.0, *T. armillata* – on the bark of *Abies holophylla* and *Quercus mongolica* with median pH 5.4, and *T. titanica* – on the bark of *Chosenia arbutifolia*, *Populus maximowiczii*, and some other trees with median pH 6.9.

IDENTIFICATION KEY

As our work has expanded the *Trichia botrytis* complex from 7 to 16 taxa, and all new species are absent in the previously published keys, we provide a diagnostic key to the *Trichia botrytis* complex and allied species.

Key to the *Trichia botrytis* complex and allied species

1 Sporocarps sessile or very short stalked. Sporotheca tuber-shaped, slightly flattened and sometimes subglobose. Dehiscence lines not contrasting, with blurry margins.

Spore ornamentation close to baculate, spores about 11.5–13.0 μm ..... ***Trichia rapa*** (grass litter at the burnt area)

1* Sporocarps almost always stalked, seldom on short stalks. Sporotheca usually obovate, sometimes subglobose. Dehiscence lines distinct or not. Spore ornamentation pilate or baculate **2**

2 Outer peridium always or often with outgrowths in a form of dark papillae or warts or with greenish-yellow spots made of wax-like substance **3**

2* Surface of the outer peridium smooth, rough, or finely tuberculous, but without any specific outgrowths **4**

3 Sporocarps usually lower than 0.8 (–1) mm. Peridium plates with blurry margins or hardly developed, with noticeable warts or papillae in the center of every plate. Elaters branched. Spiral bands decorated with distinct spinules .... ***Hemitrichia pardina*** (bark of living trees, litter, wood)

[see also ***H. minor***, which sometimes (but not necessary) bears thickenings on peridium, but differs in smaller sporocarps and type of capillitium ornamentation]

3* Sporocarps usually higher than 1 mm. Peridium plates dark, polygonal, with very contrasting boarders and noticeable papillae in the center of every plate. Elaters simple. Spiral bands decorated with minute warts .... ***Trichia papillata*** (dung, litter, wood)

3** Sporocarps always higher than 1 mm. Peridium plates usually dark, with uneven boarders and multiple greenish-yellow spots of wax-like substance on the surface. Elaters simple, with gradually tapering tips .... ***Trichia botrytis*** **var.** ***cerifera*** (wood)

4 Spiral bands decorated with prominent pointed spines ... **5**

4* Spiral bands decorated with minute warts and thickenings, so the elater surface seems rough or somewhat velvety (use oil immersion to study this feature). Tips of elaters short, not exceeding 20 μm **8**

4** Spiral bands do not bear any warts or spines, although sometimes spirals may be flattened and look sharp on the periphery **9**

5 Capillitium and spores in mass from dull orange to brick-red. Peridium plates indistinct or hardly noticeable .... ***Hemitrichia paragoga*** (wood)

[see also some species of ***Metatrichia***, that occasionally have poorly-developed peridium plates]

5* Capillitium and spores in mass from yellowish-brown to bright yellow. Peridium plates well developed **6**

6 Capillitium reticulate, without free ends, decorated with abundant spines up to 8 μm long, spores 8–10 μm .... ***Hemitrichia spinifera*** (wood)

6* Capillitium of simple or rarely branched elaters, with free ends **7**

7 Elaters simple, with tips 4–8 μm long, decorated with abundant spines up to 2 μm long. Spores 12–13 μm .... ***Trichia erecta*** (wood)

7* Elaters simple and branched, with tips 10–12 μm long and expanded nodules, decorated with spines 5–12 μm long. Spores 7–8 μm ..... ***Trichia nodosa*** (bark)

8 Peridium plates dark. Elaters branched, 3 μm wide, with tips 7–9 μm long. Spores 7–9 μm .... ***Hemitrichia velutina*** (bark of living trees with pH 3–4)

8* Peridium plates light. Elaters simple, ca. 4.3–5.7 μm wide, with tips 8–17 μm long. Spores 9–12 μm .... ***Trichia musicola*** (litter of *Musa* spp. with pH ~7)

9 Spores very small, usually no more than 9 μm **10**

9* Spores medium or large, usually bigger than 9 μm **12**

10 Spores dark brown in mass, spores 7.5–9.0 μm, sporotheca up to 0.5 μm wide ... ***Trichia microspora*** (wood, litter)

10* Spores yellow or light yellow in mass, sporotheca wider than 0.5 μm **11**

11 Peridium plates with blurry margins or practically not developed. Elaters without prominent secondary ornamentation, with tips ca. 44 μm long. Spores usually larger than 8 μm ..... ***Trichia gradalia*** (wood)

11* Peridium plates with distinct margins. Elaters with secondary ornamentation, with tips 10–12 μm long. Spores usually smaller than 8 μm. Capillitium may include nodes, decorated with spines 5–12 μm long ..... ***Trichia nodosa*** (bark)

12 Elaters with secondary ornamentation of longitudinal striae or small-meshed net between spirals (use oil immersion to study this feature) **13**

12* Elaters without prominent secondary ornamentation **19**

13 Pitch of spiral bands large (at least in some cases), spiral bands loose. Secondary ornamentation reticulate **14**

13* Pitch of spiral bands usually small, spiral bands densely arranged. Secondary ornamentation of longitudinal or slightly oblique striae; small reticulate areas are visible only under SEM **15**

14 Spiral bands left-handed ..... ***Trichia taeniifila*** (wood)

14* Spiral bands right-handed .... ***Trichia taeniifila*** (wood)

15 Tips of elaters very short, shorter than 20 μm **16**

15* Tips of elaters medium, longer than 20 μm **17**

16 Stalk short, usually not exceeding 40 % TH. Elaters 6–7 μm wide, tips of elaters 13–18 μm long. Spores not exceeding 11 μm ..... ***Trichia nubila*** (wood)

16* Stalk long, usually exceeding 40 % TH. Elaters 4–5 μm wide. Tips of elaters 4–8 μm long. Spores more than 11 μm ..... ***Trichia erecta*** (wood) (spineless form)

17 Elaters not exceeding 6 μm wide .... ***Trichia pinicola*** (bark of living trees with pH~4)

17* Elaters wider than 6 μm **18**

18 Sporocarps gregarious, but not densely. Elaters very long, sometimes longer than 1 (–4) mm. Tips of elaters 30 μm long ..... ***Trichia acetocorticola*** **var.** ***acetocorticola*** (bark of living trees with pH 4–5)

18* Sporocarps in dense colonies. Elaters long, but not exceeding 1 mm. Tips of elaters 44 μm long .... ***Trichia acetocorticola*** **var.** ***aggregata*** (bark of living trees with pH ~4)

19 Inner peridium distinctly warted (seen by LM) **20**

19* Inner peridium almost smooth, with rods, lines or mosaic pattern, but never evenly warted **21**

20 Sporocarps tiny, 120–200 μm high. Elaters very short, 70–120 μm long. Spores dull yellow in mass. Strictly corticolous species ..... ***Trichia titanica*** (bark of living trees with pH ~7)

20* Sporocarps small, 170–320 μm high. Elaters medium, 200–370 μm long. Spores bright yellow in mass. Mainly inhabiting litter, rarely may be encountered on bark .... ***Trichia flavicoma*** sensu auct. (litter, sometimes bark of living trees)

21 Strictly corticolous species. Peridium plates scarce, about 3–5 pcs. Capillitium 170–290 μm long. Tips of elaters 23 μm long ..... ***Trichia armillata*** (bark of living trees with pH ~5.5)

21* Species may occasionally inhabit bark. Peridium plates usually more than 5 or hardly developed **22**

22 Species can be encountered on litter, rarely on wood or bark **23**

22* Species usually encountered on rotten wood **24**

23 Sporocarps usually small, lower than 0.8 mm. Stalk usually not exceeding 40 % TH ..... ***Trichia flavicoma*** (litter, less commonly bark or wood)

23* Sporocarps usually large, up to 1.5 (–1.8) μm. Stalk usually long, 50–70 % TH ..... ***Trichia munda*** (litter, less commonly bark or wood)

24 Tips of elaters short, 10–15 μm long. Peridium plates not pronounced ..... ***Trichia subfusca*** (wood)

24* Tips of elaters medium, 30–50 μm long. Peridium plates abundant, but their margins are blurry and sometimes practically indistinct. Peridium usually of light color ..... ***Trichia ambigua*** (wood)

24** Tips of elaters long, 70–100 μm long. Peridium plates not abundant. Peridium usually of dark color .... ***Trichia botrytis*** (wood)

CONCLUSIONS

Comprehensive analysis of 158 specimens belonging to the *Trichia botrytis* complex resulted in the expansion of the genus *Trichia* from 30 to 39 species. Fifteen species, six of which have been previously described, are thoroughly analyzed and provided with nucleotide sequences of 2–3 marker genes. The diagnostic characteristics and their taxonomic significance are also discussed. As a result, we consider the most important aspects of detailed examination/revision/description of specimens belonging to the family Trichiaceae in general, and the genus *Trichia* in particular, to be the availability of: 1) high-quality color images of the sporocarp habit and size; 2) measurements of elater length (in case they are simple), length of elater tips, elater width, and spore diameter, with the accuracy of 0.1 μm for the latter two; 3) data on the ornamentation of inner peridium, capillitium, and spores, ideally using SEM; 4) information on ecological preferences, including substrate pH in case of moist chambers.

Phylogenetic analysis revealed that the family Trichiaceae is extremely heterogeneous, and species of the genera *Trichia*, *Hemitrichia*, *Metatrichia*, and *Oligonema* are intermixed. We hope that the accumulation of linked morphological and molecular data will allow to derive a more coherent system in the future and find morphological and ecological evidence for the forthcoming revisions based on phylogenetic analysis.

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