FUSE

Fungal Systematics and Evolution

VOLUME 17
JUNE 2026
PAGES 110–123

doi.org/10.15761/fuse.2026.17.07

A five-finger exercise: five overlooked non-European species of *Tubifera*

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In memoriam Yukinori Yamamoto (1947–2025), a prominent mycologist and leading authority on Japanese myxomycetes.

Keywords:
Amoebozoa
Eumycetozoa
Myxogastrea
new taxa
Reticulariaceae
Taxonomy

Abstract: A critical revision of the Herbarium of the National Museum of Nature and Science (Tsukuba, Japan) and several other collections has resulted in the description of five new myxomycete species of the genus *Tubifera*. These include *T. annulifera* and *T. digitella* from Japan, *T. coralloides* from Australia and New Zealand, *T. yamamotoi* from Japan, the USA, and the Seychelles, and *T. suavis* from the USA, Japan, France, and Kamchatka. Three of these species were previously known only from a few unidentified barcodes. A phylogeny mainly based on nucSSU sequences showed that *T. annulifera* and *T. coralloides* are related to *T. ferruginosa*, *T. digitella* is a sister species to *T. montana*, while *T. yamamotoi* together with *T. suavis* form a clade with *T. dimorphotheca*. Species delimitation using the distance-based ASAP and branch-length based PTP methods confirmed the distinctness of the newly discovered taxa and also supported the elevation of *T. ferruginosa* subsp. *acutissima* to the rank of an independent species *T. acutissima*. We also describe two sections within the genus *Tubifera*, sect. *Tubifera* and sect. *Microtubifera*, and provide new molecular barcodes for *Siphoptychium casparyi*.

Citation: Leontyev DV, Kochergina AV, Inoue M, Lloyd SJ, Schnittler M (2025). A five-finger exercise: five overlooked non-European species of *Tubifera*, related to *T. dimorphotheca, T. ferruginosa*, and *T. montana*. *Fungal Systematics and Evolution* **17**: 110–123. doi: 10.15761/fuse.2026.17.07
Received: 5 December 2025; **Accepted:** 29 March 2026; **Effectively published online:** 10 April 2026
Corresponding editor: P.W. Crous

INTRODUCTION

The genus *Tubifera*, described in 1792, is one of the most recognizable taxa of myxomycetes. Its finger-like sporocarps are pinched together forming dense clusters, the so-called pseudoaethalia, which resemble a wasp nest or an insect egg case. The species content of this genus has been changing in a rather peculiar way. In the 18th and 19th centuries, about twenty species were described, but most of them turned out to be synonyms of the most common species, *T. ferruginosa*. By the mid-20th century, seven species were recognized within the genus, three of which – *T. bombarda*, *T. casparyi,* and *T. dictyoderma* – were later transferred to three other genera (*Alwisia*, *Siphoptychium*, and *Thecotubifera,* respectively (Leontyev *et al*. 2014, 2019). One of the four remaining "true" *Tubifera* species, *T. papillata*, is known only from the type collection, while the other three species, *T. ferruginosa*, *T. microsperma*, and *T. dimorphotheca,* are well documented in biodiversity surveys. These species differ in spore size (the spores in *T. microsperma* are smaller than those of the others), in the formation of a distinct hypothallic stalk (in *T. microsperma* and *T. dimorphotheca*), and in the presence of spherical sporothecae covering the stalk (in *T. dimorphotheca*) (Martin & Alexopoulos 1969, Nannenga-Bremekamp 1991, Poulain *et al*. 2011).

A critical revision of the group began with a reassessment of the diagnostic criteria used to delimit species. Nelson *et al*. (1982) showed that the inner surface of the peridium in *T. microsperma* bears rimmed craters, whereas in *T. ferruginosa* this surface is completely smooth. The application of this criterion made it possible to justify two additional species, *T. applanata* with a ring-ornamented peridium and *T. dudkae* with a surface sculptured by wavy folds (Leontyev 2009, Leontyev & Fefelov 2012, Leontyev & Moreno 2011). These species also differed in the shape of the sporothecae and the topography of the pseudoaethalial surface, thereby expanding the range of morphological traits used for species diagnosis in the genus.

The introduction of molecular methods contributed to further progress in the study of the group. DNA barcoding based on a fragment of the 18S rRNA gene (nucSSU) not only confirmed the distinctness of the previously described species but also enabled the description of eight additional ones (Leontyev *et al*. 2019, 2015, Lloyd *et al*. 2019, Vlasenko *et al*. 2024). The system of diagnostic criteria was enriched by such characters as the colour of peridial iridescence, the degree of fusion of sporothecae, the shape of their apices, the pigmentation of immature fruiting bodies etc. In less than twenty years, the number of recognized species in *Tubifera* has increased from four to fourteen. The new species have gained general acceptance, were included in identification keys (Gmoshinskiy *et al*. 2021, Yamamoto 2021), and are well represented in biodiversity databases (Leontyev & Yatsiuk 2024, Yatsiuk *et al*. 2024).

In the most comprehensive compilations of molecular barcodes (Leontyev *et al*. 2019, 2015), in addition to the identified species, three more were named as *Tubifera* sp. The limited number and often poor condition of these specimens did not allow for a reliable assessment of their morphology, and the authors postponed formal description until more material could be studied. This opportunity arose through access to the collection of the National Museum of Nature and Science (Tsukuba, Japan), which houses numerous *Tubifera* specimens. Additional material from various countries was collected by authors of this paper or obtained from Z. Edwards (USA), T.I. Kryvomaz (Ukraine), S.J. Lloyd (Australia), M. Meyer (France), Y.K. Novozhilov (Russian Federation), B. Sheehan (USA), S.L. Stephenson (USA), and S. Young (Australia). The results of this study are presented below.

MATERIALS AND METHODS

The study involved 85 myxomycete specimens, 83 of which were examined morphologically and molecularly by the first author at different times, while DNA sequences for the remaining two were retrieved from GenBank. Within the current research we conducted the morphological study of 45 specimens, collected in Japan (23 samples), USA (7), Australia (5), Kamchatka Peninsula (5), South Korea (2), France (1), New Zealand (1) and Seychelles (1). These specimens were also studied molecularly, if their barcodes were not obtained in previous studies. In addition, we used DNA extracts from 16 more collections, kept at the University of Greifswald, to obtain mtSSU sequences (see below). Altogether 57 collections were molecularly barcoded within this research, and 75 sequences were newly obtained (Supplementary File 1).

Since some of the specimens we analysed were assigned, based on morphological characters, to the rare *Tubifera dimorphotheca*, we compared them with the type material of this species, preserved in the herbarium of the Meise Botanic Garden in Belgium (NENB10675).

All morphological examination and imaging of myxomycete specimens were performed using a Keyence VHX-7000 digital microscope and a Leica DM2500 microscope equipped with a Flexacam C1 camera. Scanning electron microscopy was carried out with a Zeiss Evo LS10 device in the Institute of Microbiology, University of Greifswald. Size variation is presented in the descriptions as (minimum) 25% percentile – 75% percentile (maximum) for pseudoaethalia, and as (minimum) Mean−SD – Mean+SD (maximum) for spores (see Supplementary File 2). Spore size measurements were rounded to two significant digits, down when closer to the lower bound, up when closer to the upper bound, with the last digit adjusted to 0 or 5.

We followed the methods of DNA extraction and amplification described in our recent paper (Leontyev *et al*. 2025b). For all studied specimens we sequenced two independently inherited markers: partial sequences of the nuclear (nucSSU) and mitochondrial (mtSSU) genes of the small subunit ribosome RNA (Leontyev *et al*. 2022). Obtained sequences were deposited in the NCBI GenBank under accession numbers PX649528–PX649602. The data for the third marker, the gene of eukaryotic elongation factor 1 alpha DNA (*EF1a*), was taken from the previous publication (Leontyev *et al*. 2019). Molecular barcodes for four specimens of *Cribraria* and four of *Dianema*, used as outgroups, were retrieved from the supplementary data of Zamora *et al.* (2025).

Individual alignments of SSU genes were compiled with MAFFT v. 7 (Katoh *et al*. 2019) using automatic selection of the alignment method. The protein-coding *EF1a* sequences were aligned in MACSE v. 2.04 (Ranwez *et al.* 2011). The resulting alignments comprised 120 (nucSSU), 48 (mtSSU) and 20 (*EF1a*) sequences, including outgroups. These data were combined using the Concatenator software (Vences *et al*. 2022) with the following partitioning: nucSSU 1–1556, mtSSU 1557–2102, *EF1a* 2103–2795 (Supplementary File 3).

For the construction of the maximum likelihood tree based on the combined three-gene alignment the IQtree webserver was used (Nguyen *et al.* 2015). The best evolutionary model chosen by the ModelFinder (Kalyaanamoorthy *et al.* 2017), was SYM+I+G4 for nucSSU, GTR+F+G4 for mtSSU, TPM3u+F+I+G4, TN+F+I and TIM2+F+G4 for codon positions of *EF1a*. The Shimodaira-Hasegawa approximate likelihood ratio test, the approximate Bayes test and a Bootstrap analysis with 1000 pseudo-replicates were used as criteria of the branch support.

Preliminary species delimitation was carried out separately for nucSSU and mtSSU using the genetic distance based method Assemble Species by Automatic Partitioning (ASAP) at the platform iTaxoTools (Vences *et al.* 2022) employing the Kimura (K80) 2.0 distance model (Puillandre *et al.* 2021). Five best scores were calculated for each marker. Alternative partitioning based on branch lengths in the three-gene tree was performed using the single-threshold Poisson Tree Processes (PTP) algorithm in the PTP application for Windows (Zhang *et al.* 2013), with a Newick tree rooted on the longest branch.

The supplementary data for this paper are available on Zenodo: 10.5281/zenodo.18343533.

RESULTS

Our phylogeny based on 120 nucSSU, 48 mtSSU and 20 *EF1a* sequences (Fig. 1) showed that the studied specimens are distributed among 12 branches within the genus *Tubifera* and two within the genus *Siphoptychium*. Sixteen of these specimens clustered within *T. ferruginosa*, *T. montana*, *T. glareata*, *T. tomentosa*, *T. vanderheuliae, S. casparyi*, and *S. reticulatum*. Five specimens were assigned to *T. dimorphotheca* based on morphological data, including the analysis of the type collection. The remaining specimens formed six clusters not corresponding to any described taxon. The PTP partition, based on the three-gene phylogeny, indicated that all six groups are sufficiently distinct to be considered separate species. This result was corroborated by the distance-based method ASAP, performed separately for nucSSU and mtSSU sequences (Supplementary File 4). Even with the data being still fragmentary for mtSSU and *EF1a*, the enumeration of the genetic variants for each marker (Supplementary File 4) shows that there are no intergroup recombination (Shchepin *et al*. 2022): every species of *Tubifera* is characterised by an own set of genetic variants. Based on these data, we herein describe five of these groups as the new species *T. annulifera*, *T. coralloides*, *T. digitella*, *T. suavis*, and *T. yamamotoi*. The sixth species, *T. "bruxnerensis"*, is represented by a single, poorly preserved specimen, and we currently refrain from describing it.

Molecular data analysis using species delimitation methods indicated that *T. ferruginosa* subsp. *acutissima*, previously described as a subspecies (Leontyev *et al*. 2015), is sufficiently distinct from *T. ferruginosa* to be considered a separate species. This is supported by PTP and ASAP results for nucSSU and mtSSU, as well as by the position of subsp. *acutissima* with respect to *T. ferruginosa* (Fig. 1). We formally publish the new status for this taxon below.

Our phylogeny showed that species of the genus *Tubifera* group into two major clades (Fig. 1, Supplementary File 5), previously described as "large-spored" and "small-spored" (Lloyd *et al*. 2019). We now treat these clades as the sections *Tubifera* and *Microtubifera*. The species *T. magna* and *T. tomentosa*, forming a third, most basal branch on the genus tree, are not assigned to either section, since (1) this clade shows low statistical support, (2) only single gene data are available for both species of this clade, and (3) no clear morphological characters support its separation.

Taxonomy

Tubifera J.F. Gmel., *Syst. nat.*, *ed. 13* (Leipzig), **2**(2): 1472. 1792.

Tubifera* sect. *Tubifera

Diagnosis: This section unites species with larger spores, on average bigger than 6 µm.

Description: Pseudoaethalia of various shape, mainly pulvinate. Hypothallus may be rather conspicuous (*T. acutissima*, *T. dudkae*), but never forms a stalk. Sporothecae uniform, cylindrical, erect, rarely sinuous, bag-like, or spherical (*T. dudkae*). Peridium iridescent in blue and green or golden and pink (*T. montana*, *T. digitella*) colours. Inner surface of the peridium ornamented by wavy folds and less prominent rings. Spores reticulate, (5.5–)6.0–8.5(–10.5) µm diam.

Typus: *Tubifera ferruginosa* (Batsch) J.F. Gmel., *Syst. nat., ed. 13* (Leipzig), **2**(2):1472. 1792.

Species: *T. acutissima*, *T. annulifera, T. coralloides, T. digitella, T. dudkae, T. ferruginosa, T. glareata, T. montana, T. vanderheuliae.*

Tubifera* sect. *Microtubifera Leontyev & Schnittler, ***sect. nov.*** MB 861498.

Etymology: *micro* (Greek) small, referring to the smaller spores.

Diagnosis: Section unites species with smaller spores, on average smaller than 7 µm.

Description: *Pseudoaethalia* of various shape, bundle- or fascicle-like, pulvinate, stalked or sessile. Hypothallus may form a stalk, which elevates pseudoaethalium above substrate (*T. dimorphotheca*, *T. microsperma*, *T. pseudomicrosperma*). *Sporothecae* often represented by two types: cylindrical and spherical, the latter situated at the base of pseudoaethalium (*T. corymbosa*, *T. yamamotoi*) or covering the hypothallic stalk (*T. dimorphotheca*); in other cases, such spherical sporothecae are absent. *Peridium* dull, or weakly iridescent on sporothecal apices (*T. corymbosa*). Inner surface of the peridium ornamented by prominent rings or craters, but wavy folds may occur as well (*T. suavis*). *Spores* reticulate, (4.0–)5.0–6.5(–7.5) µm diam.

Typus: Tubifera microsperma (Berk. & M.A. Curtis) G.W. Martin, *Mycologia* **39**(4): 461. 1947.

Species: *T. applanata, T. corymbosa, T. dimorphotheca, T. khangaiensis, T. microsperma, T. suavis, T. yamamotoi.*

Species *incertae sedis*: *T. magna, T. tomentosa.*

Tubifera acutissima (Leontyev *et al*.) Leontyev, Schnittler & S.L. Stephenson, ***comb. & stat. nov.*** MB 861497.
Basionym: Tubifera ferruginosa subsp. *acutissima* Leontyev *et al*., *Mycologia* **107**(5): 965. 2015. MB 809787.

Typus: **USA**, Virginia, mixed mesophytic forest, 37.219550 N, 80.329110 W, 1189 m.a.s.l., decaying wood covered with bryophytes, 23 Jun 2013, *S.L. Stephenson* (**holotype** UARK 50546).

Pseudoaethalia solitary or grouped, 4–25 mm long, short ovoid or irregular as observed from above, pulvinate to hemispherical, often forming moniliform complexes, rust-brown, yellowish brown. *Sporothecae* cylindrical, rounded in cross section, straight, directed from the base to the external surface of the fructification, 0.3–0.5 mm diam. *Apices* of sporothecae not accreted, conical, acute conical, with pointed ends. *Hypothallus* spongy, white when fresh, yellowish when mature. *Peridium* semitransparent, light brown in reflected light, slightly iridescent with blue, purple and golden tints. External surface of the peridium verrucose. Internal surface of peridium smooth. *Columella, capillitium* and *pseudocapillitium* absent. *Spores* (5.5–)6.1–7.0(–7.5) µm in diam., rusty-brown in mass, light yellowish in transmitted light, reticulated. Immature fructifications deep pink, later turning almost black.

Comment: We provide a full description of *T. acutissima* here, since the original publication included only a short diagnosis and a colour plate (Leontyev *et al*. 2015, p. 967). As stated in the diagnosis, this taxon differs from *T. ferruginosa* s. str. (previously *T. ferruginosa* subsp. *ferruginosa*) by the shape of the apices (tips) of sporothecae, which are acute conical and pointed. This shape is very characteristic, easily observable even in the field, and has no close analogy within the genus. Although other species, like *T. ferruginosa* s. str. and especially *T. annulifera*, may have narrowed, nearly conical tips, they are never pointed or drawn out at the ends. Another character that distinguishes *T. acutissima* is the pigmentation of young fructifications, that are deep pink, rather than red, orange or flesh-coloured, as in most of *Tubifera* species.

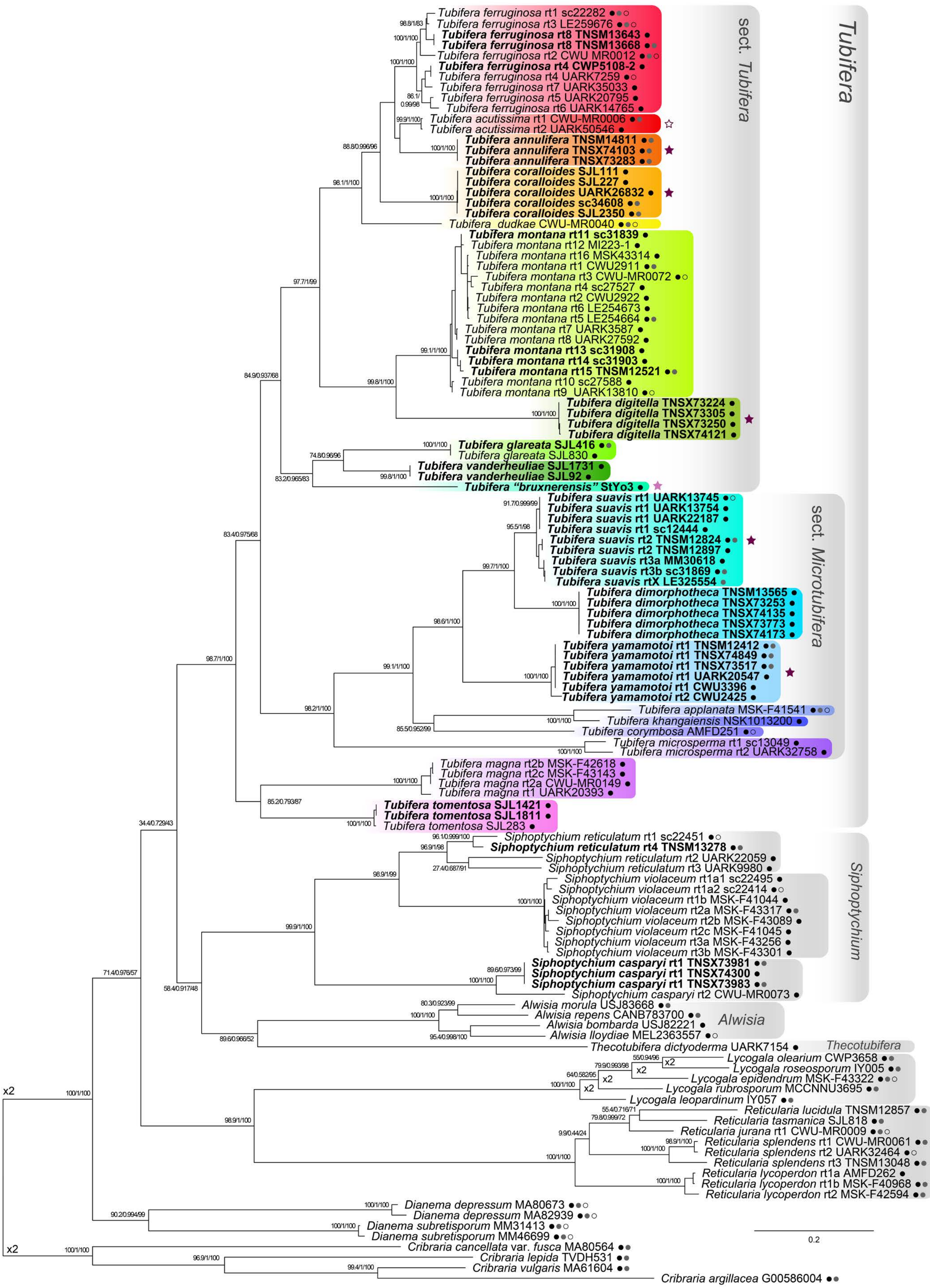

Fig. 1. Maximum likelihood phylogeny of the Reticulariaceae, based on 120 nucSSU, 48 mtSSU, and 20 EF1a sequences. Branch support is shown as follows: Shimodara-Hasegawa SH-aLRT test / Approximate Bayes test / Ultrafast bootstrap test (1000 replicates). Bold: specimens studied here. Dark star: species described here; light star: species yet undescribed; contoured star: subspecies elevated to species status. Dots: available sequences, black – nucSSU, grey – mtSSU, white – *EF1a*.

Tubifera annulifera Leontyev, Y. Yamamoto, Schnittler, ***sp. nov.*** MB 861492. Fig. 2.

Etymology: *annulus* (Lat.), ring, *ferre* (Lat.) bear; referring to the ring-like area, surrounding the tip of the sporotheca.

Diagnosis: Differs from *T. ferruginosa* by silvery, pointed but not elongated sporothecal apices, surrounded by the iridescent, ring-like area.

Pseudoaethalia solitary or grouped, 5–20 mm long, rounded, ovoid or irregular as observed from above, pulvinate to hemispherical, dull brown (Fig. 2A). *Sporothecae* cylindrical, rounded in cross section, directed from the base to the external surface of the fructification, 0.3–0.5 mm diam. (Fig. 2B–E). *Apices* of sporothecae not accreted, blunt conical, visually separated from the rest of sporothecae by the iridescent ring (Fig. 2D, E). *Hypothallus* spongy, inconspicuous. *Peridium* semitransparent, light brown in reflected light, slightly iridescent with blue, purple and golden tints, with the apical part appearing silvery (Fig. 2D, E). External surface of the peridium verrucose (Fig. 2 F, G). Internal surface of peridium smooth (Fig. 2H). *Columella, capillitium* and *pseudocapillitium* absent. *Spores* (5.5–)6.0–7.0(–7.5) µm diam., rusty-brown in mass, light yellowish in transmitted light. About 2/3 of the spore surface is covered by a regular reticulum with 5–6 meshes across the diameter, the rest is ornamented with irregularly branched bands which form an intricate pattern (Fig. 2I, J). *Immature fructifications* not observed.

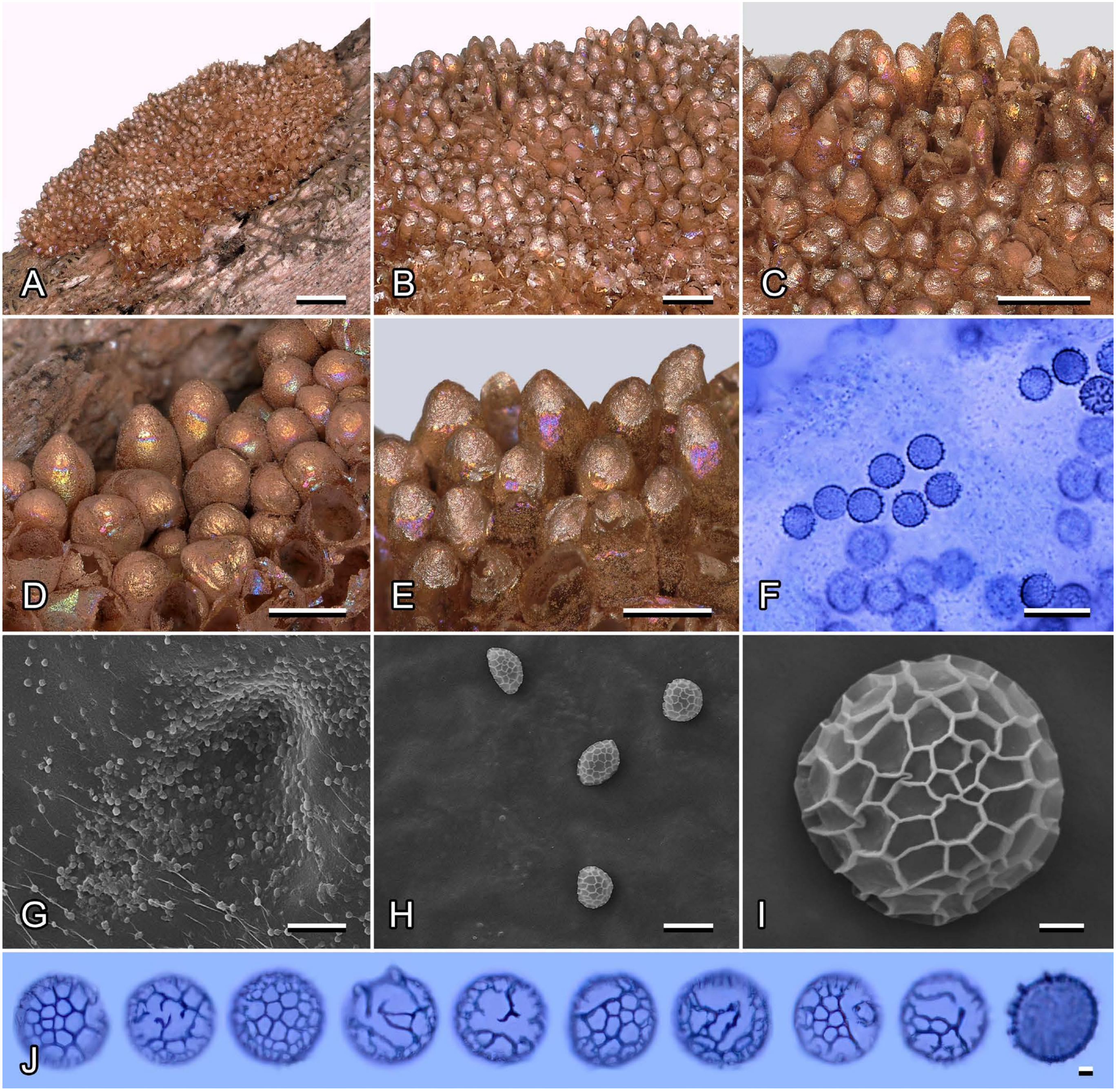

Fig. 2. *Tubifera annulifera* sp. nov. **A, B.** Pseudoaethalium. **C–E.** Apices of sporothecae. **F.** Peridium and spores, stained by methyl blue in lactic acid. **G.** Outer surface of the peridium, SEM. **H.** Inner surface of the peridium, SEM. **I.** Spore, SEM. **J.** Spores, stained by methyl blue in lactic acid; the last one is shown in optical section. Specimens: A–C, E, G–I. TNSX73283 (Holotype). D. TNSM14811. F. TNSX74103. J. TNSX74103 (1–2), TNSM14811 (3–10). Scale bars: A = 2 mm. B, C = 1 mm. D, E = 0.5 mm. F = 10 µm. G, H = 5 µm. I, J = 1 µm. Photo authorship: D.V. Leontyev.

Typus: **Japan**, Kochi, Motoyama-cho, Takatsuno, 33.70995, 133.53609, on decayed wood, 16 Jul. 2008, *leg.* Yukinori & Yoko Yamamoto (**holotype** TNSX73283). GenBank: *nucSSU* = PX649561, mtSSU = PX649536.

Distribution: Asia (so far known only from Japan).

Comment: This species is a close relative and a "twin" of *Tubifera ferruginosa* and *T. acutissima*. According to our phylogeny (Fig. 1), it is a sister species of the latter. The most distinctive feature of *T. annulifera* is the iridescent ring, mentioned in the diagnosis, which separates the apex of the sporotheca from its main cylindrical part. Although the type of iridescence is known to be relatively stable in Myxomycetes (Poulain *et al*. 2011), and in *Tubifera* in particular (Leontyev *et al*. 2015), the stability of this character in *T. annulifera* requires further study.
We examined 24 specimens assigned to the genus *Tubifera* from the collection of the Museum of Nature and Science, Tsukuba, using molecular methods. Among them, there were only two samples that turned out to be *T. ferruginosa*, and both of these had been collected in South Korea, not on the Japanese Islands. Of course, such a limited collection is insufficient to cast doubt on the presence of *T. ferruginosa* in Japan. However, it may be assumed that this species, which is one of the most common in the forests of the Holarctic, is at least not frequent in Japan. It cannot be ruled out that at least some records of *T. ferruginosa* reported from Japan may in fact represent *T. annulifera*.

Tubifera coralloides Leontyev, S.J. Lloyd, S.L. Stephenson, M. Schnittler, ***sp. nov.*** MB 861493. Fig. 3.

Etymology: *corallium* (Lat.), coral; referring to the coral-like branching of sporothecae and the coral red color of developing fructifications.

Diagnosis: Differs from the other *Tubifera* species by having rounded outgrowths on the sporothecal apices, which resemble coral branches.

Pseudoaethalia in pairs, small groups or solitary, 7–10 mm long, rounded or short ovoid as observed from above, pulvinate to hemispherical, dull brown (Fig. 3B, C). *Sporothecae* irregularly cylindrical to vermiform, rounded in cross section, directed from the base to the external surface of the fructification, 0.3–0.6 mm diam. (Fig. 3D, E). *Apices* of sporothecae closely appressed but not accreted, forming short coral-like branches with knobby, irregularly rounded or flattened ends (Fig. 3E). *Hypothallus* spongy, usually inconspicuous, or visible as a mass of white strands at the base of the pseudoaethalium. *Peridium* semitransparent, light brown in reflected light, dull or slightly iridescent with blue, purple and golden tints (Fig. 3D, E). External surface of the peridium verrucose (Fig. 3G). Internal surface of peridium ornamented with rings 0.5–3 µm diam. (Fig. 3F, H). *Columella, capillitium* and *pseudocapillitium* absent. *Spores* (5.0–)6.0–7.5(–8.0) µm diam., rusty-brown in mass, light yellowish in transmitted light. About half of the spore surface is covered by a regular reticulum with 7–8 meshes across the diameter, the rest is ornamented with irregularly branched bands which form an intricate pattern (Fig. 3I, J) or a large-meshed reticulation (Fig. 3J, spore 4). *Immature fructifications* coral red (Fig. 3A).

Typus: **Australia**, New South Wales, Gold Coast, Mountain Retreat, Dorrigo NP, Never-Never Picnic area, on decayed wood, −30.35999, 152.80034, 25 Feb 2023, leg. *M. Schnittler et al.* (**holotype** sc34608). GenBank: *nucSSU* = PX649563, mtSSU = PX649539.

Distribution: continental Australia, Tasmania, New Zealand.

Comment: Despite the considerable diversity in the pseudoaethalial surface structure in *Tubifera*, nothing resembling the morphology of *T. coralloides* has been observed until now. The coral-like branching of the sporothecal apices is a unique feature that allows this species to be identified even in the field.
For the three specimens of *T. coralloides* collected in Tasmania, the substrate is known − dead wood of *Banksia marginata*. This is rather unusual, since the other three species of *Tubifera* found in Tasmania (*T. glareata*, *T. tomentosa*, *T. vanderheuliae*) develop on dead eucalyptus wood (Lloyd *et al*. 2019). Substrate preferences may serve as an additional diagnostic feature of the new species.

Tubifera digitella Leontyev, Y. Yamamoto, Schnittler, ***sp. nov.*** MB 861494. Fig. 4.

Etymology: *digitellus* (Lat.) little finger; referring to the shape of sporothecae.

Diagnosis: Differs from *T. montana* by weakly fused or nearly free sporothecae, shaped like fingers or sausages.

Pseudoaethalia solitary or grouped, 2–15 mm long, rather irregular as observed from above, pulvinate to bunch-like, bright rusty-brown with rather conspicuous iridescence (Fig. 4A–C). *Sporothecae* nearly free or slightly attached to each other, cylindrical, sausage-like, rounded in cross section, directed from the base to the external surface of the fructification, 0.6–0.8 mm diam. (Fig. 4D, E). *Apices* of sporothecae free, convex (Fig. 4D, E). *Hypothallus* spongy, consisting of whitish strands of dried slime. *Peridium* semitransparent, light brown in reflected light, iridescent with golden and purple tints (Fig. 4D, E). External surface of the peridium slightly verrucose to nearly smooth (Fig. 4G). Internal surface of peridium is at least partially ornamented with wavy folds, forming a kind of reticulum (Fig. 4F). *Columella, capillitium* and *pseudocapillitium* absent. *Spores* (6.0–)6.5–8.0(–8.5) µm diam., rusty-brown in mass, light yellowish in transmitted light. Most of the spore surface is covered by a regular reticulum with 7–8 meshes across the diameter; the reticulation-lacking area indistinct, with less regular, ruptured reticulation (Fig. 4I, J). *Immature fructifications* not observed.

Typus: **Japan**, Kochi, Konan-shi, Yasu-cho, Hao, 33.59690, 133.83353, on decayed wood, 12 Jul. 2008, leg. *Yukinori et al*. (**holotype** TNSX73224). GenBank: *nucSSU* = PX649567.

Distribution: Asia (so far known only from Japan).

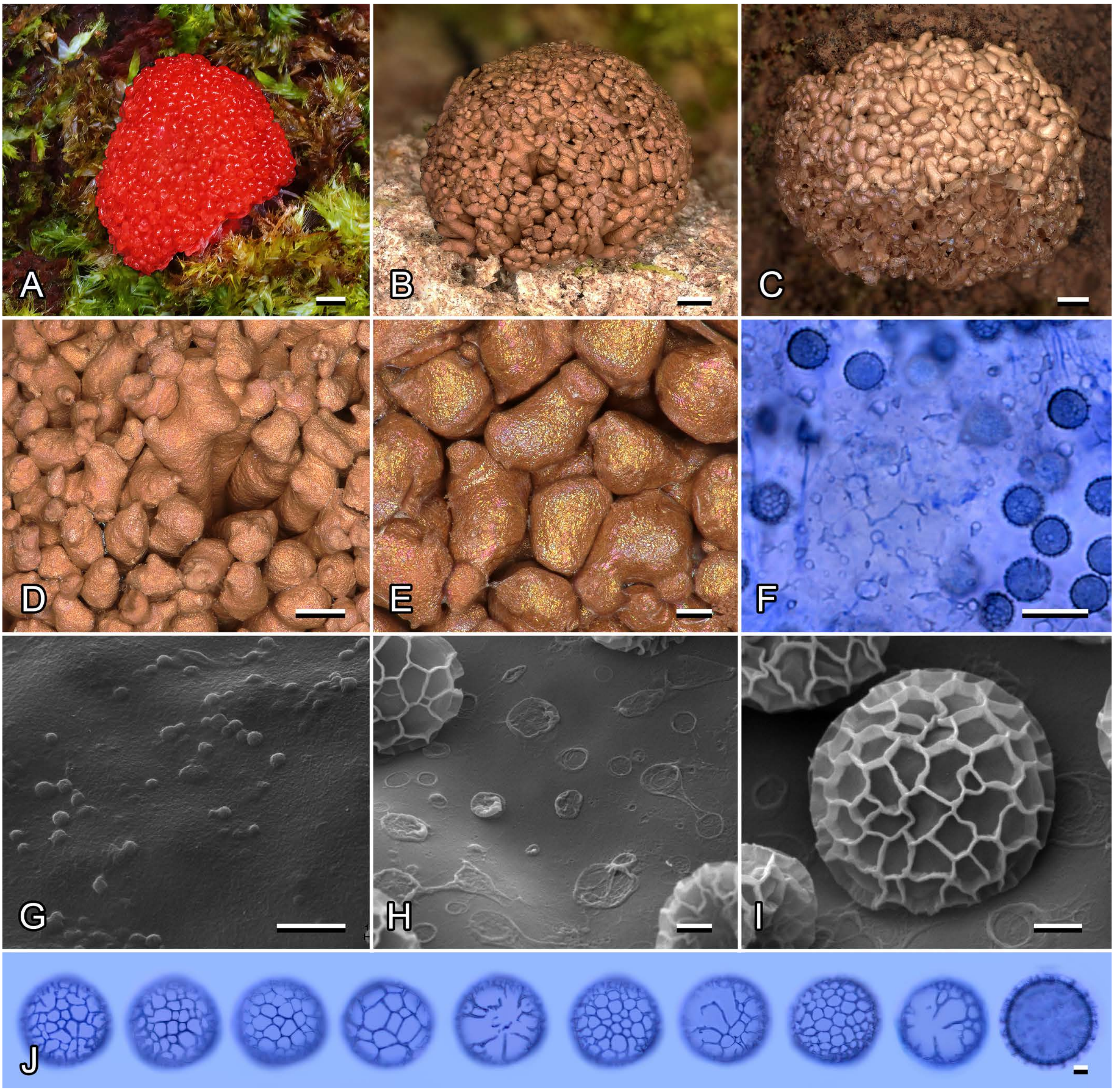

Fig. 3. *Tubifera coralloides* sp. nov. **A.** Immature pseudoaethalium. **B, C.** Pseudoaethalia. **D, E.** Apices of sporothecae. **F.** Peridium and spores, stained by methyl blue in lactic acid. **G.** Outer surface of the peridium, SEM. **H.** Inner surface of the peridium, SEM. **I.** Spore, SEM. **J.** Spores, stained by methyl blue in lactic acid; the last one is shown in optical section. Specimens: A. Not collected. B, D–I. sc34608 (Holotype). B. SJL2350. J. sc34608 (1–2), SJL2350 (3–10). Scale bars: A–C = 1 mm. D = 0.5 mm. E = 0.2 mm. F = 10 µm. G = 5 µm. H–J = 1 µm. Photo authorship: A. S.J. Lloyd. B–J. D.V. Leontyev.

Comment: According to our phylogeny (Fig. 1), this charming myxomycete is a sister species of *T. montana*. Both taxa share several morphological features, including the vivid golden-pink iridescence of the peridium and the relatively large diameter of the sporothecae (up to 1 mm). However, *T. digitella* differs markedly in that its sporothecae are arranged very loosely and often appear as separate fruiting bodies rather than parts of a pseudoaethalium. This feature is not entirely unique, but in combination with other characters it allows for the reliable identification of *T. digitella*.

Tubifera suavis Leontyev, Schnittler, S.L. Stephenson, M. Kobayashi, M. Meyer, Y.K. Novozhilov, O.N. Shchepin, ***sp. nov.*** MB 861495. Fig. 5.

Etymology: *suavis* (Lat.), pleasant; referring to the neat sporothecal apices.

Diagnosis: Differs from *T. montana* by the absence of pink and golden iridescence, and from *T. magna* by small and convex pseudoaethalia.

Pseudoaethalia mainly grouped, 5–20 mm long, rather irregular as observed from above, pulvinate, dull brown (Fig. 5A, B). *Sporothecae* cylindrical, slightly polygonal in cross section, directed from the base to the external surface of the fructification, 0.5–1 mm diam. (Fig. 5C, D). *Apices* of sporothecae closely accreted to each other, convex, polygonal, forming a tessellate ornament on the surface of

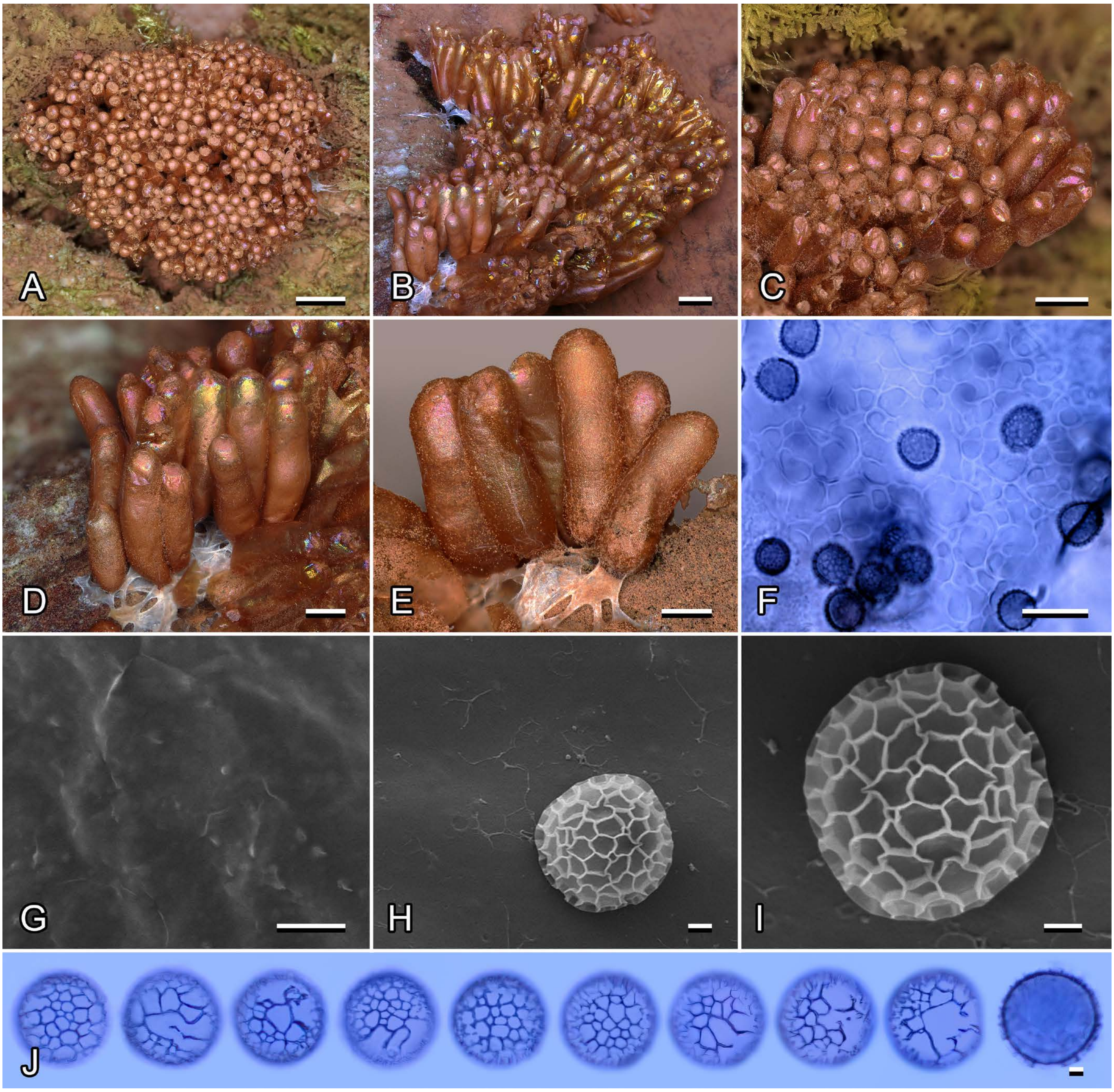

Fig. 4. *Tubifera digitella* sp. nov. **A–C.** Pseudoaethalia. **D, E.** Apices of sporothecae. **F.** Peridium and spores, stained by methyl blue in lactic acid. **G.** Outer surface of the peridium, SEM. **H.** Inner surface of the peridium and the spore, SEM. **I.** Spore, SEM. **J.** Spores, stained by methyl blue in lactic acid; the last one is shown in optical section. Specimens: A, C, G–I. TNSX73224 (Holotype). B, D–F. TNSX73250. J. TNSX73224 (1–5), TNSX73250 (6–10). Scale bars: A = 2 mm. B, C = 1 mm. D, E = 0.5 mm. F = 10 µm. G = 5 µm. H–J = 1 µm. Photo authorship: D.V. Leontyev.

pseudoaethalium (Fig. 5D); in American collections apices free, rounded (Fig. 5E). *Hypothallus* spongy, consisting of whitish strands of dried slime. *Peridium* semitransparent, light brown in reflected light, dull or faintly silvery (Fig. 5C–E). External surface of the peridium verrucose (Fig. 5G). Internal surface of peridium is ornamented with wavy folds, forming a kind of reticulum (Fig. 5F, H). *Columella, capillitium* and *pseudocapillitium* absent. *Spores* (5.5–)6.0–7.0(–7.5) µm diam., rusty-brown in mass, light yellowish in transmitted light. About 2/3 of the spore surface is covered by a regular reticulum with 7–10 meshes across the diameter; the reticulation-lacking area indistinct, with less regular, ruptured reticulation (Fig. 5I, J). *Immature fructifications* not observed.

Typus: **Russia**, Kamchatka Krai, Milkovskiy District, 12 km SSE from village Lazo, at the foot of the northern slope of Nikolka Volcano, valley of the Cheremoshnaya River, 55.45083, 159.79662, on a strongly decayed log of *Larix cajanderi* 70 cm in diam., 20 Aug 2019, leg. *Y.K. Novozhilov et al.* (**holotype** sc31869). GenBank: *nucSSU* = PX649589, *mtSSU* = PX649551.

Distribution: Asia (Japan, Kamchatka), Europe (France), North America (USA).

Comment: According to molecular data, *T. suavis* is related to *T. dimorphotheca* and *T. yamamotoi*, and these two are characterized by the formation of abortive spherical sporothecae at the base of the pseudoaethalium. However,

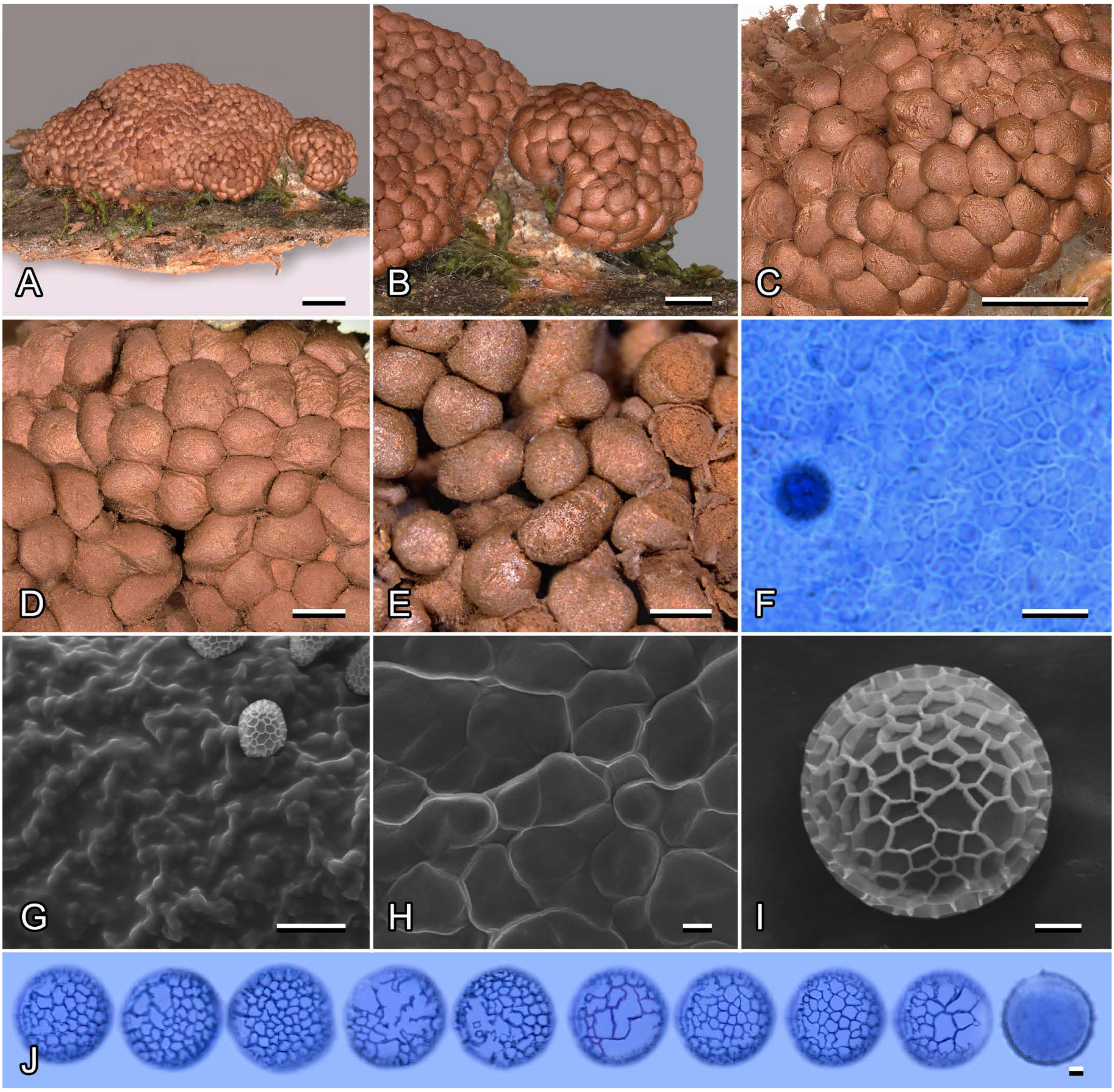

Fig. 5. *Tubifera suavis* sp. nov. **A, B.** Pseudoaethalia. **C–E.** Apices of sporothecae. **F.** Peridium, stained by methyl blue in lactic acid. **G.** Outer surface of the peridium, SEM. **H.** Inner surface of the peridium and the spore, SEM. **I.** Spore, SEM. **J.** Spores, stained by methyl blue in lactic acid; the last one is shown in optical section. Specimens: A–C, G–I. sc31869 (Holotype). D. LE325554. E. UARK22187. F. TNSM12824. MM30618 (1–5), sc12444 (6–10). Scale bars: A = 2 mm. B, C = 1 mm. D, E = 0.5 mm. F = 10 µm. G = 5 µm. H–J = 1 µm. Photo authorship: D.V. Leontyev.

in nine specimens of *T. suavis* originating from three continents, we did not observe any spherical sporothecae. This species is nevertheless similar to its relatives in the overall appearance of the fruiting bodies. First, the peridium is matte and rather lightly coloured. Second, the sporothecae along the periphery of the fruiting body are characteristically smoothed, giving the entire pseudoaethalium rounded contours.

A problematic feature of this species is the considerable variation in the degree of fusion of the sporothecal apices. In Japanese and Kamchatkan specimens, tight fusion predominates, whereas in North American specimens the apices are arranged more loosely (Fig. 5E). This morphological variability is also reflected at the genetic level, as specimens from the Old and New Worlds form two subclades (Fig. 1). Species delimitation using ASAP based on mtSSU even suggests that *T. suavis* may represent two distinct biological species. However, the number of investigated specimens is too low for a statistically sound recombination test (see Shchepin *et al.* 2022); and other delimitation methods do not support this hypothesis.

Tubifera yamamotoi Leontyev, T. Kryvomaz, H. Hagiwara, B. Sheehan, ***sp. nov.*** MB 861496. Fig. 6.

Etymology: Named after Yukinori Yamamoto, Japanese myxomycetologist, collector of the holotype of the species.

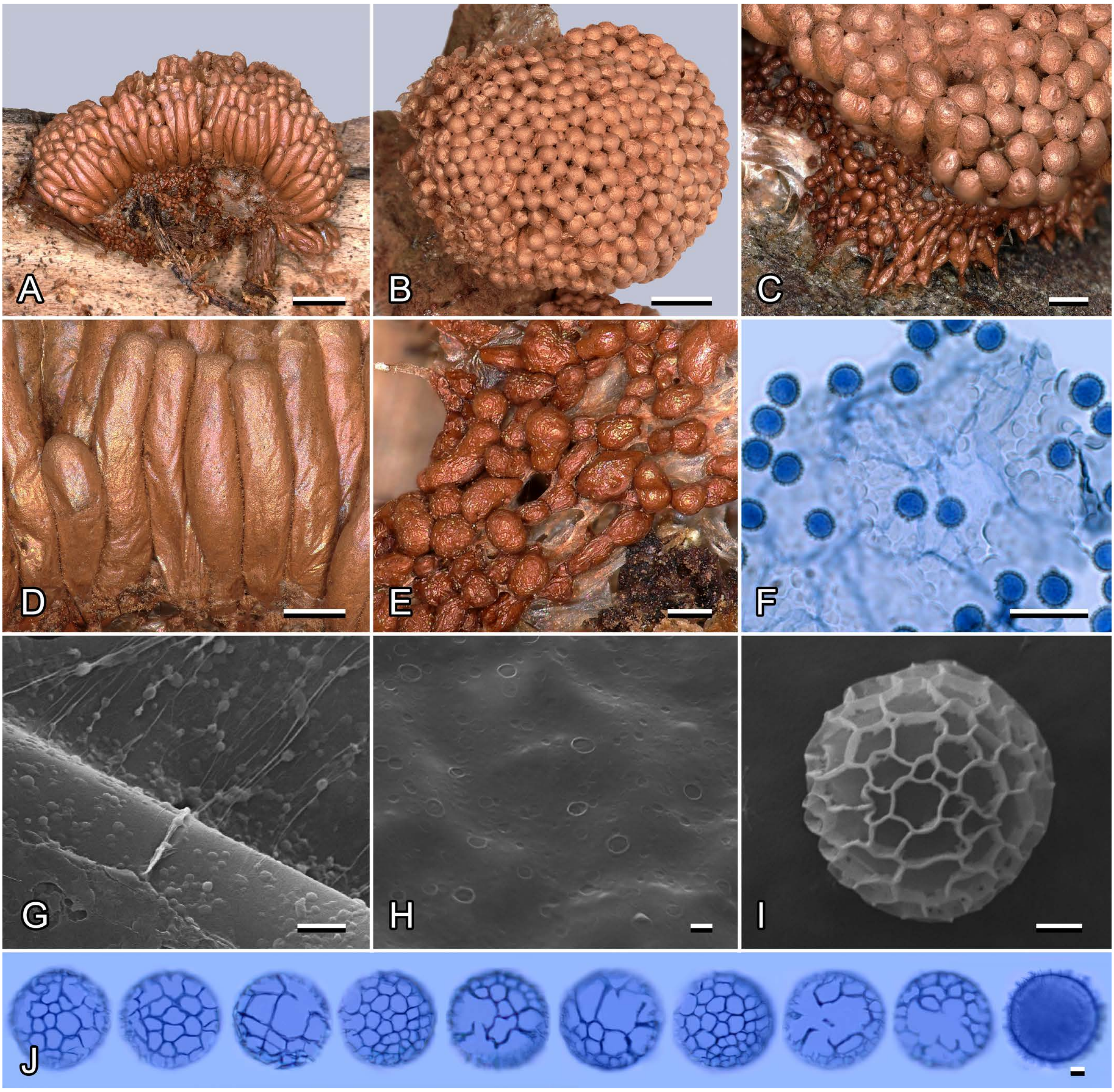

Fig. 6. *Tubifera yamamotoi* sp. nov. **A, B.** Pseudoaethalia. **C.** Cylindrical (above) and spherical (below) sporothecae. **D.** Cylindrical sporothecae. **E.** Spherical sporothecae on the hypothallus of the pseudoaethalium. **F.** Peridium and spores, stained by methyl blue in lactic acid. **G.** Outer surface of the peridium, SEM. **H.** Inner surface of the peridium and the spore, SEM. **I.** Spore, SEM. **J.** Spores, stained by methyl blue in lactic acid; the last one is shown in optical section. Specimens: A, C–G, I. TNSX73517. B. TNSX12412. H. CWP2925. J. TNSX73517 (1–2), TNSM12412 (3–10). Scale bars: A, B = 2 mm. C, D = 0.5 mm. E = 0.2 mm. F = 10 µm. G = 5 µm. H–J = 1 µm. Photo authorship: D.V. Leontyev.

Diagnosis: Differs from *T. dimorphotheca* by wide-spread, sessile pseudoaethalia, having no differentiated stalk.

Pseudoaethalia grouped, 5–20 mm long, rounded or short ovoid as observed from above, pulvinate, light brown (Fig. 6A, B). *Cylindrical sporothecae* light brown, cylindrical, rounded in cross section, directed from the base to the external surface of the fructification, 0.4–0.5 mm diam. (Fig. 6D). *Apices* of these sporothecae loosely attached to each other, with small interstices between them (Fig. 6B). *Spherical sporothecae* dark rusty-brown, irregularly rounded, closely covering the hypothallus below the cylindrical sporothecae (Fig. 6C, E). *Hypothallus* spongy, consisting of whitish strands of dried slime. *Peridium* semitransparent, light brown in reflected light, dull or slightly iridescent (Fig. 6 D). External surface of the peridium verrucose (Fig. 6G). Internal surface of peridium is nearly smooth, ornamented with faint rings 0.5–1 µm diam. (Fig. 6H) and/or with wavy folds, forming a kind of reticulum (Fig. 6F); the letter type of ornamentation seems to be rare. *Columella, capillitium* and *pseudocapillitium* absent. *Spores* (5.0–)5.5–7.0(–7.5) µm diam., rusty-brown in mass, light yellowish in transmitted light. About 2/3 of the spore surface is covered by a regular reticulum with 6–7 meshes across the diameter; the reticulation-lacking area indistinct, with less regular, ruptured reticulation (Fig. 6 I, J). *Immature fructifications* not observed.

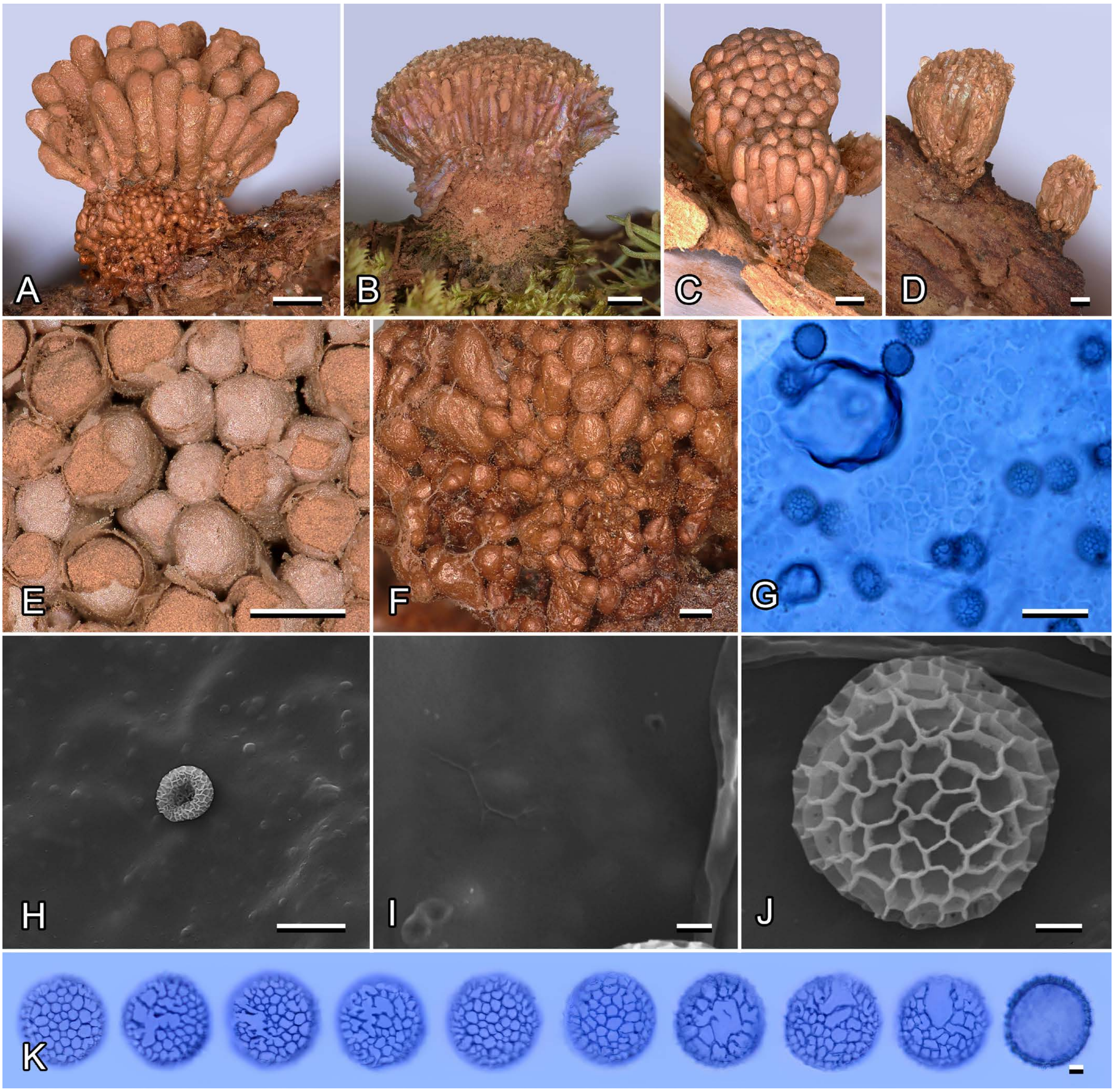

Fig. 7. *Tubifera dimorphotheca.* **A–D.** Pseudoaethalia. **E.** Apices of cylindrical sporothecae. **F.** Spherical sporothecae on the hypothallic stalk of the pseudoaethalium. **G.** Peridium and spores, stained by methyl blue in lactic acid. **H.** Outer surface of the peridium with a collapsed spore, SEM. **I.** Inner surface of the peridium, SEM. **J.** Spore, SEM. **K.** Spores, stained by methyl blue in lactic acid; the last one is shown in optical section. Specimens: A, E, F, H–J. NENB10675 (Type). B, D. TNSM13565. C. MM9907. D, G. TNSX73253. K. NENB10675 (1–5), TNSX73253 (6–10). Scale bars: A, B = 1 mm. C–E = 0.5 mm. F = 0.2 mm. G = 10 µm. H = 5 µm. I–K = 1 µm. Photo authorship: D.V. Leontyev.

Typus: **Japan**, Kochi, Motoyama-cho, Kinodu, 33.75158, 133.59630, on decayed wood, 3 Sep. 2008, leg. *Yukinori & Y. Yamamoto* (**holotypus** TNSX73517). GenBank: *nucSSU* = PX649600, *mtSSU* = PX649554.

Distribution: Asia (Japan), Africa (Seychelles), North America (USA).

Comment: This species is closely related to *T. dimorphotheca* (Fig. 7) and shares the most characteristic feature of the latter: the presence of abortive spherical sporothecae, which cover the hypothallus. However, as stated in the diagnosis, *T. yamamotoi* is easily distinguishable by the absence of hypothallic stalk, which elevates the bunch of sporothecae above the substrate. Spores of *T. dimorphotheca*, based on the holotype we studied (NENB10675), seem to be somewhat smaller than in *T. yamamotoi*: (5.1–)5.7–6.4(–6.9) µm vs (5.2–)5.6–6.6(–7.2) µm. The spore ornamentation differs as well: the number of meshes per hemisphere is 8–10 vs 6–7. Another similar species, the Central American *T. corymbosa* (Leontyev *et al*. 2015) differs from *T. yamamotoi* by the bunchy shape of pseudoaethalia and silvery lids on the sporothecal apices.

DISCUSSION

With the new additions, the genus *Tubifera* is now represented by 20 species, most of which seem to have limited distributions (Leontyev & Yatsiuk 2024). Even widespread species such as *T. ferruginosa*, *T. montana*, and *T. magna* are apparently not cosmopolitan: their distribution is restricted to the Holarctic, and in all three species the European and American collections are represented by different genetic variants. *T. applanata*, *T. dudkae*, and probably *T. pseudomicrosperma* are Eurasian endemics; *T. corymbosa* occurs in Central America; *T. glareata*, *T. tomentosa*, and *T. vanderheuliae* inhabit Australia. *T. microsperma*, according to GBIF data, is distributed throughout the tropics. However, the African and Asian records of this species have never been barcoded, and the American specimens, according to the taxon delimitation inferred using ASAP and PTP methods (Supplementary File 4), seem to include two different biological species.

Five new Tubiferas complement this picture. *T. coralloides* inhabits Australia and Oceania; *T. annulifera* and *T. digitella* appear to be endemics of Japan, or at least of East Asia; *T. yamamotoi* and *T. suavis* occur in Asia and North America, with the former also recorded from the Seychelles and the latter known from a single European occurrence. *T. acutissima*, previously described as a subspecies, likely has a Holarctic range. It is noteworthy that the classical *T. ferruginosa*, previously reported from all continents except Antarctica, was not found by us in Japan and Australia − all collections turned out to belong to the new species described here.

The results obtained expand our understanding of myxomycete endemism in Australia and Japan. *T. coralloides* has been found in Tasmania, eastern Australia, and New Zealand, within a triangle of Gondwanan regions already known as a hotspot of the myxomycete diversity (Lloyd 2022, Lloyd *et al*. 2024b). Three *Tubifera*, two *Alwisia*, and one *Reticularia* species have already been described from this region (Leontyev *et al*. 2025b, Lloyd *et al*. 2024a). *Tubifera coralloides,* as well as yet undescribed *T. "bruxnerensis"* (Fig. 1), represent other taxa belonging to this relictic biota.

Endemism of the Japanese myxomycete biota is less well known, although there is a rather extensive list of species occurring only or predominantly there. *Siphoptychium casparyi* and *Thecotubifera dictyoderma* (both are members of *Reticulariaceae*) belong to such taxa. Recently the list of probable Japanese endemics was supplemented by a species of *Reticularia* − *R. lucidula* (Leontyev *et al*. 2025b). And now this list has been extended by four more *Tubifera* species, two of which have been found only on Japanese islands.

Tubifera dimorphotheca, a well-known and thoroughly described but rather rare species, warrants a separate discussion. Currently, three molecular barcodes attributed to this species are listed in the GenBank. However, we now understand that the presence of abortive spherical sporothecae at the base of the pseudoaethalium is not a unique feature of this taxon. This raises the question of whether the barcodes deposited in the GenBank indeed correspond to this species. We were able to examine the type specimen of *Tubifera dimorphotheca* housed in Meise, (NENB10675) and conclude that five specimens from Japan belong to *T. dimorphotheca* (see Fig. 7). However, the barcodes we obtained from these collections do not match those currently represented in the GenBank. One of the sequences previously deposited in this database by D.V. Leontyev, CWU M3396 (MK396665), belongs to *T. yamamotoi*. The specimen AMFD251 (JX481345) was previously identified as *T. corymbosa* (Leontyev *et al.* 2015). The third sequence (ON969986.1) is very short and, according to BLAST results, belongs to Spirochetes. Thus, the five sequences of *T. dimorphotheca* obtained in this study may represent the first more or less reliably identified barcodes of this species. Naturally, full certainty would require barcoding of the type specimen itself, which necessitates applying more modern sequencing techniques and is of considerable interest to us.

In this study we used of three independent species delimitation algorithms: (1) ASAP, based on genetic distances, (2) PTP, based on branch lengths, and (3) recombination analysis, based on the biological species concept. These were applied to analyse the molecular data for two independently inherited non-Mendelian genes, nucSSU and mtSSU (the third gene, *EF1a*, was not studied in the new species, with one exception). All three delimitation methods show a very similar pattern of species borders and support the separation of the new species described here. However, some inconsistencies were found. The ASAP based on mtSSU divides *T. suavis* into two species, while PTP and nucSSU-based ASAP recognise only one. The widely distributed and genetically variable *T. ferruginosa* is treated as a single species by PTP, but two ASAP analyses, based on two different genes, separate it into several species, and their delimitation differs strongly (see Supplementary File 4). The similar pattern of contradiction between nucSSU and mtSSU data was observed in *Lycogala*, where delimitation of *L. epidendrum* based on these two genes is hardly comparable (Leontyev *et al.* 2025a). In both *T. ferruginosa* and *L. epidendrum* we may observe the speciation in progress: regional populations of widely distributed species start to accumulate genetic differences, which may lead (or already led) to the formation of new species. In the "grey zone" of speciation, different lines of evidence may strongly contradict each other (de Queiroz 2007, Degnan *et al.* 2009, Mirarab *et al.* 2021), and this is likely the case we observe. Much more data are needed to understand whether some genetic variants of *T. ferruginosa* and *L. epidendrum* deserve to be formally described as separate species.

In addition to *Tubifera* we also barcoded four specimens of *Siphoptychium* from the collection of the Museum of Nature and Science in Tsukuba. Three of them turned out to be *S. casparyi* and one *S. reticulatum* (see Fig. 1). For the former species, these are the first reliable molecular data confirming its distinctiveness. In the 2019 publication in which the genus *Siphoptychium* was re-erected, we had only one fresh specimen of *S. casparyi*, collected in the Ural Mts. It was severely damaged, and we expressed doubts about its identity (Leontyev *et al*. 2019). The new data (Fig. 1) show that the Ural specimen is close but not identical to the true *S. casparyi* from Japan, and may represent a separate species (Supplementary File 4). Similarly, all four genetic variants of *S. reticulatum*, including the Japanese one discovered by us, according to ASAP and PTP represent separate species. More material is needed to describe them.

In our previous publications, we did not examine species-specific features of spore ornamentation in different species of *Tubifera*, since this required a staining technique we developed later (Leontyev *et al.* 2023a). The new data show that the general ornamentation pattern appears to be uniform across the genus: all studied species have a small and poorly expressed reticulation-free area, sometimes completely covered by a fragmented network. This contrasts sharply with the ornamentation in *Lycogala* and especially in *Reticularia* (Leontyev *et al.* 2025a, b). However, species-level differences are still traceable, especially concerning the relative size of the meshes, a character that is known to be taxonomically significant in Reticulariaceae (Leontyev *et al*. 2023b). Further research is required to reveal ornamentation patterns in other species of the genus.

In this paper, we finally decided to describe the sections of big-spored and small-spored *Tubifera*, the existence of which we had previously postulated (Lloyd *et al*. 2019). However, in earlier works we relied on a single-gene nucSSU phylogeny, which separated all species of the genus into two clades. In our new phylogeny, two species, *T. magna* and *T. tomentosa*, form a basal group that we do not assign to any sections because mtSSU data for these taxa are lacking (Fig. 1). Interestingly, the spores of these two species have an intermediate size, compared to the large spores of sect. *Tubifera* and the small spores of sect. *Microtubifera* (Supplementary File 5). However, no other evidence supports the third clade, and its two species do not show obvious morphological similarities. Therefore, we refrain from formally describing this clade.

The results of this study show that a careful analysis combining molecular data with thorough morphological investigation makes it possible to detect and describe even rare and hardly distinguishable species. To paraphrase a well-known aphorism, the mills of integrative taxonomy grind slowly, but exceedingly fine.

ACKNOWLEDGEMENTS

This study was supported by a MSCA4Ukraine grant (1208119) awarded to the first author. Lab procedures were in part supported by a grant within the DFG graduate school “Response” (RTG2010) to the fifth author. We are deeply grateful to Dr Tsuyoshi Hosoya (National Museum of Nature and Science, Tsukuba, Japan) for his assistance in locating and selecting herbarium specimens. Our special appreciation goes to Myriam de Haan (Meise Botanic Garden, Belgium) for kindly providing access to the important type specimen of *Tubifera dimorphotheca*. We are indebted to all collectors whose material was used in this study, including numerous non-institutional researchers, some of whom wish to remain anonymous.

This project has received funding through the MSCA4Ukraine project, which is funded by the European Union. Views and opinions expressed are however those of the authors and do not necessarily reflect those of the European Union, the European Research Executive Agency or the MSCA4Ukraine Consortium. Neither the European Union nor the European Research Executive Agency, nor the MSCA4Ukraine Consortium as a whole nor any individual member institutions of the MSCA4Ukraine Consortium can be held responsible for them.

Conflict of Interest: The authors declare no conflicts of interest.

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Supplementary Material: http://fuse-journal.org/

Supplementary File S1. Collection and barcoding data for all studied specimens of *Tubifera* and *Siphoptychium.* New taxa and GenBank data are shown in bold (*xlsx* file).

Supplementary File S2. Spore measurement of for the studied specimens of *Tubifera.* A. Spore size of individual specimens. B. Spore size of the species of *Tubifera*. C. Box and whisker plot showing spore size range in specimens of *Tubifera* (*xlsx* file).

Supplementary File S3. Concatenated alignment of partial nucSSU (positions 1–1556), mtSSU (1557–2102), and EF1a (2103–2795) sequences (*fasta* file).

Supplementary File S4. Preliminary species delimitation based on ASAP, PTP and recombination analyses. For the PTP and ASAP, each number correspond to the delimited species. For the recombination data, each number for every gene corresponds for the genotype. For ASAP, for each of two genes the five partitions are shown, with the partition score value (line 3) and the number of delimited species (line 4). The partitions with the best (lowest) score are marked in red (*xlsx* file).

Supplementary File S5. Spore size of *Tubifera* species in different sections of the genus (*xlsx* file).