THE GENUS *FISSIDENS* IN RUSSIA, I: SPECIES OF *FISSIDENS MONGUILLONII* AFFINITY

РОД *FISSIDENS* В РОССИИ, I: ВИДЫ ИЗ РОДСТВА *FISSIDENS MONGUILLONII*

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Abstract

A preliminary molecular phylogenetic analysis of *Fissidens* species of the *F. bryoides*-complex revealed one species with a relatively weak uni- to bistratose libmidium nested in a clade in which almost all the samples have stout multistratose limbidia. It is a well-defined species, relatively widely distributed in European Russia, in scattered localities eastwards to the Urals. In its nuclear ITS DNA sequences it is most similar to *F. monguillonii* from Altantic Europe. However, it is a much smaller plant, resembling small phenotypes of *F. viridulus*, and unlike *F. monguillonii* it has a weak uni- or bistratose limbidium. This *Fissidens* species is described as a new species, *F. mosquensis*. The clade formed by *F. monguillonii* and *F. mosquensis* further includes the East Asian *F. lateralis* (=*F. bryoides* var. *lateralis*), a species also characterized by a multistratose limbidium and perichaetial leaves narrower than stem leaves. *Fissidens mosquensis* is compared with *F. bryoides* and several morphotypes of *F. viridulus* s.l. A terrestrial form of *F. rivularis* from the Caucasus with a uni- to bistratose leaf margin is also discussed. Plants with this combination of traits are nested in a clade in which the other samples represent aquatic *F. rivularis*.

Резюме

Предварительный молекулярно-филогенетический анализ видов комплекса *Fissidens bryoides* позволил выявить один вид с листьями, имеющими по краю сравнительно слабую 1–2-слойную кайму, который оказался в кладе, почти все представители которой имеют мощную многослойную кайму. Это хорошо морфологически отграниченный вид, довольно широко распространенный в европейской части России, от Смоленской области до Урала. По нуклеотидным последовательностям ядерного участка ITS он ближе всего к *F. monguillonii,* распространенному в атлантических районах Европы, но, в отличие от последнего вида, это намного более мелкое растение со слабой 1–2-слойной каймой. Он описан как новый для науки вид, *F. mosquensis*. Клада *F. monguillonii* и *F. mosquensis* включает также восточноазиатский вид *F. lateralis* (=*F. bryoides* var. *lateralis*), для которого также характерны листья с многослойной каймой и более узкие по сравнению со стеблевыми перихециальные листья. Дано сравнение *Fissidens mosquensis* с *F. bryoides* и несколькими морфотипами *F. viridulus* s.l. Также обсуждается наземная форма *F. rivularis* с Кавказа, имеющая листья с не характерной для этого вида 1–2-слойной каймой, которая по данным анализа ДНК принадлежат той же в кладе, к которой относятся и типичные водные образцы *F. rivularis* с многослойной каймой.

KEYWORDS: bryophytes, mosses, taxonomy, molecular barcoding, ITS, *trn*S-F, new species

INTRODUCTION

Recent advances in phylogenetic reconstructions have improved the infrafamilial and infrageneric classifications of many difficult moss genera, such as *Grimmia* (Hernández-Maqueda *et al*., 2008,) *Racomitrium* (Larraín *et al*., 2013), *Schistidium* (Ignatova *et al*., 2010), and *Didymodon* (Jiménez *et al*., 2022).

The genus *Fissidens* has remained little studied until recently (Guerra *et al*., 2021; Suzuki *et al*., 2018, Budke *et al*., 2022). These authors have sufficiently elucidated the general phylogeny of the genus.

At the same time, the taxonomy of some puzzling groups in the genus remains variously interpreted by different authors. One of the most difficult is a species complex of rather small plants with limbate leaves, related to *Fissidens bryoides* Hedw. These species are common in hemiboreal and temperate forests across the Holarctic. Recent floras in Europe have accepted rather narrowly defined species (Bruggeman-Nannenga, 1986; Guerra & Ederra, 2015; Smith, 2004), thus following traditional European treatments (Limpricht, 1890; Warnstorf, 1904; Brotherus, 1923). In North America, most authors have

synonymized most morphotypes under the *F. bryoides* complex (Pursell, 1976, 2007a, 2007b). Crum & Anderson (1981) likewise accepted *F. bryoides sensu lato*, but provided a key and illustrations for several infraspecific taxa. Japanese authors accepted five subspecies in *Fissidens bryoides* (Iwatsuki & Suzuki, 1982), each with a detailed description, and the same approach to the taxonomy of this complex was used for the Moss Flora of China (Li & Iwatsuki, 2001).

In Russia different names have been applied stochastically by different authors, and thus the data compiled in the checklist of East Europe and North Asia (Ignatov *et al.*, 2006) comprised species distributions based on publications with non-correlated approaches. Therefore, this group is in need of thorough revision.

In the course of a study of the genus *Fissidens* in Russia we used ITS sequence markers and found great diversity, which is more or less structured in supported clades. These clades include samples characterised by similar geographic distributions and some morphological distinctions. A complete description of this diversity is under preparation for publication elsewhere. Here we will discuss mainly one species of this group represented by several specimens from European Russia. It can be keyed out as *F. monguillonii* using the key in Smith (2004). At the same time, the description given by Smith (l.c.) differs from those given in the Moss Flora of the Iberian Peninsula (Guerra & Ederra, 2015), illustrated by Lüth (2019), and discussed by Bruggeman-Nannenga (1986) and Bailly (2010). Any discussion on this species must necessarily address a group of species near *F. rivularis*, which is usually considered easy to key out due to growth in aquatic or subaquatic habitats and having thickened multistratose limbidium. In relation to the newly recognised species we also discuss the diversity of the *Fissidens bryoides* complex and species which can be confused with it, e.g. *F. gymnandrus* and *F. viridulus*.

Materials and Methods

Molecular studies. One nuclear and one plastid region were selected for analysis based on previous studies: these are the nuclear internal transcribed spacer region, ITS (including ITS1, gene 5.8S RNA and ITS2) and the plastid region *trn*S-F (including *trn*S-*rps*4-*trn*T-*trn*L-*trn*F cluster, with four tRNA genes (*trn*S (partial), *trn*T, *trn*L, *trn*F (partial)), a fast evolving gene (*rps*4), four spacers separating the coding regions, as well as one group I intron). These markers have been sequenced for many moss taxa, and have proved to be informative in various moss families (e.g., Olsson *et al.*, 2009a; Ignatov et al., 2019; Fedosov et al., 2022). We sequenced *trn*S-F for one sample of each species, while many others possess a shorter fragment of *trn*L-F. The molecular studies followed the laboratory protocol for DNA extraction, amplification and sequencing described in, e.g., Gardiner *et al.* (2005) for ITS and *trn*L-F, and in Olsson *et al.* (2009a) for *trn*S-F. Sequences were aligned using MA-

FFT v. 7.505 (2022/Apr/10) with E-INS-i aligning strategy with otherwise default options.

Bayesian analysis was performed separately for the nuclear ITS and plastid *trn*S-F/*trn*L-F datasets. The analyses were performed in MrBayes 3.2.6 (Ronquist *et al.*, 2012) with the GRT+G model, and run with 5 000 000 generations (reaching all PSRF equal to 1.000, and ESS>1000). Partitioning followed the initially suggested partitions, nruns=4, nchain=6, temp=0.02.

Evolutionary analysis by Maximum Likelihood was performed using General Time Reversible model (Nei & Kumar, 2000). Evolutionary analyses were conducted in MEGA X (Kumar *et al.*, 2018). Vouchers of newly sequenced specimens and GenBank accession numbers of all used sequences are listed in Table 1.

The ITS tree was rooted on *F. gracilifolius*, the sequence of which differs especially strongly from most of the other studied species of the *F. bryoides*-complex. The still more distant *F. curvatus* has expanded deletions in ITS1, and consequently the alignment of its ITS with other species proved to be problematic in the preliminary studies. However, we used *F. curvatus* as the outgroup in the alignment of the plastid region *trn*S-F.

Morphological studies. These included numerous specimens used for our preliminary studies. However, the illustrations and measurements presented here include only data from samples used in the molecular phylogenetic analysis, so as to avoid using subjectively identified samples, and to limit the discussion to the clade content rather than unconfirmed identifications. Binomial names are applied to clades where the species delimitation is relatively unequivocal, but for *F. viridulus* s.l. (i.e. samples that come out as this species according to identification keys) we supplement the binomial name *F. viridulus* with an informal additional name for each lineage. The latter names are needed for subsequent discussion of the morphological distinctions between representatives of different clades.

In addition to standard microscopic observations, peristomes were studied by SNE-4500M, coated by gold without any additional preparation. Light microscope observations were made under a stereomicroscope (Olympus SZX7) equipped with an Infinity 8-8 digital camera, and compound light microscope Olympus CX-43 with an Infinity 1-2 digital camera. Stacked micrographs using several optical sections were composed using the software package HeliconFocus 4.50 (Kozub *et al.*, 2008).

Results

As the plastid data were not available for all samples, and are much less variable compared to ITS, we present here the trees for separate datasets.

Phylogenetic tree inferred from analysis of the nuclear ITS sequences is shown in Fig. 1 and for plastid *trn*S-F data in Fig. 2. The former is fairly well resolved, and many of its clades are well supported. The variation in the plastid marker is low, with solitary substitutions, and

0.05

0.94/- — viridulus 'sibiricus' OK766 Novosibirsk
0.9/- — viridulus 'sibiricus' OK3510 Khabarovsk
0.91/- — viridulus 'sibiricus' OK485 Primorsky
1/100 — viridulus 'sibiricus' OK914 Vologda
— viridulus 'sibiricus' OK352 Nizhny Novgorod
1/99
0.99/93 — viridulus 'sibiricus' OK1672 Finland
— viridulus 'sibiricus' OK484 Krasnoyarsk
— viridulus 'sibiricus' OK468 Yakutia
1/57 — viridulus 'sibiricus' OK490 Yakutia
1/- — gymnandrus OK332 Tatarstan
1/100 — gymnandrus OK965 Kostroma
— gymnandrus OK896 Ryazan
1/100 — gymnandrus OK385 Kamchatka
— arcticus OK354 Yakutia
0.96/100 — arnoldii OK469 Ryazan
1/100 — arnoldii OK470 Kaluga
1/78 — arnoldii OK2919 Moscow
— pusillus OK1671 Finland
1/100 — pusillus OK928 Murmansk
0.91/- — viridulus 'caucasicus' OK499 Dagestan
1/100 — viridulus 'caucasicus' OK 498 Kabardino-Balkaria
— viridulus 'caucasicus' OK962 Dagestan
0.91/-
0.9/- — bryoides OK900 Kostroma
— bryoides OK948 Moscow
0.92/- — bryoides MW450898 Spain
— bryoides OK344 Moscow
1/100 — bryoides OK1668 Finland
— bryoides OK329 Moscow
0.97/-
1/100 — crassipes MW450904 Spain
— crassipes OK3568 Georgia Abkhazia
— crassipes MW450906 Spain
1/100 — viridulus 'kamchaticus' OK464 Sakhalin
1/100 — viridulus 'kamchaticus' OK357 Sakhalin
— viridulus 'kamchaticus' OK386 Kamchatka
1/100 — crassipes OK3567 Ukraine
— crassipes OK479 UK Wales
0.97/- — mosquensis OK3511 Bashkiria
1/100 — mosquensis OK3555 Moscow
— mosquensis OK950 Kaluga
0.98/- — mosquensis OK952 Smolensk
0.99/67 — lateralis OK3551 Primorskiy
1/100 — monguillonii France OK3673
1/- — monguillonii France type locality OK3680
— viridulus 'orientalis' OK904 Primorsky
1/100 — viridulus 'orientalis' OK3505 Bashkortostan
1/100 — viridulus 'orientalis' OK754 Khabarovsk
— viridulus 'orientalis' OK750 Transbaikalia
— viridulus 'orientalis' OK480 Altai
1/99 — viridulus MW450910 Spain
— viridulus MW450909 Spain
0.95/- — rivularis OK3675 France
0.92/- — rivularis OK3677 France
1/100 — rivularis OK495 Krannodar
— rivularis OK3513 Georgia Adjaria
1/100 — eremicus MW450903 Spain
1/100 — eremicus MW450900 Spain
— gracilifolius OK460 Caucasus Dagestan
— gracilifolius OK726 Moscow

Fig. 1. Bayesian molecular phylogenetic tree of *Fissidens* species based on nuclear ITS sequences. Bayesian posterior probabilities >0.9 / the percentage of trees in which the associated taxa clustered together in ML analysis are shown at branches.

1/85 — viridulus 'caucasicus' OK962 Dagestan
— viridulus 'caucasicus' OK499 Dagestan
— viridulus 'orientalis' OK754 Khabarovsk
— viridulus 'orientalis' OK3505 Bashkortostan
— viridulus 'orientalis' OK750 Transbaikalia
1/70 — mosquensis 3511 Bashkortostan
0,92/- — mosquensis OK3555 Moscow
1/90 — mosquensis OK950 Kaluga
— mosquensis OK952 Smolensk
0,96/60 — arnoldii OK469 Ryazan
0,92/- — arnoldii OK470 Kaluga
0,75/- — arnoldii OK2919 Moscow
— pusillus OK928 Murmansk
0,78/- — viridulus 'kamchaticus' OK386 Kamchatka
— viridulus 'kamchaticus' OK464 Sakhalin
— viridulus 'kamchaticus' OK481 Sakhalin
0,94/60 — 1/88 — rivularis OK495 Krasnodar
— rivularis OK3513 Georgia Adjaria
— gracilifolius OK726 Moscow
0,83/- — lateralis OK3551 Primorskiy
— crassipes OK479 UK Wales
— gymnandrus OK385 Kamchatka
1/79 — gymnandrus OK965 Kostroma
— gymnandrus OK896 Ryazan
— gymnandrus OK899 Tatarstan
1/62 — viridulus 'sibiricus' OK490 Yakutia
— viridulus 'sibiricus' OK3510 Khabarovsk
1/65 — viridulus 'sibiricus' OK766 Novosibirsk
— viridulus 'sibiricus' OK485 Primorskiy
— monguillonii OK3573 France
1/98 — bryoides OK329 Moscow
— bryoides OK1668 Finland
1/100 — bryoides OK340 Moscow
— curvatus OK462 Caucasus
— curvatus OK401 Primorskiy

0.004

Fig. 2. Bayesian molecular phylogenetic tree of *Fissidens* species based on plastid *trn*S-F sequences. Bayesian posterior probabilities >0.9 / the percentage of trees in which the associated taxa clustered together in ML analysis are shown at branches.

its clades have much lower support. However, the essentially similar grouping of samples is important evidence for assessing the ITS topology, which might raise doubts because of hyper-variation and difficulties in aligning. Because of the latter difficulties we used MAFFT aligning in the phylogenetic analysis without further editing.

In the ITS-based tree (Fig. 1) the first branch splits off *F. eremicus*, and the remaining taxa form a clade with PP=1, but low support from ML analysis. This clade is further subdivided mostly into clades with low support and the maximally supported clades are mainly monospecific.

However, the topology clearly indicates that *F. viridulus* sensu lato comprises several groups which are intermingled with aquatic species with a multistratose limbidium and clades of minute saxicolous species. Several clades with more than one species were resolved in the ITS tree:

(1) *Fissidens arcticus+F. gymnandrus+F. viridulus* 'sibiricus', the latter is the most common 'molecular species' in Asian Russia, and also common in NE European Russia.

(2) *F. arnoldii+F. pusillus*

(3) *Fissidens monguillonii* from France, including a specimen from the type locality+*F. lateralis* from the Russian Far East+the 'Russian relative of *F. monguillonii*' (*'Fissidens mosquensis'* in the tree).

Other highly supported clades are monospecific, and *Fissidens crassipes* was found in two clades, one being sister to *F. bryoides* and another to *F. viridulus* 'kamchaticus'.

The tree inferred from plastid *trn*S-F (Fig.2) shows rather high support for some monospecific clades. For the clades found in the ITS-based tree, moderate support was obtained for *Fissidens arcticus+F. gymnandrus+F. viridulus* 'sibiricus' (PP=0.91).

The samples from the ITS clade of *Fissidens monguillonii+F. lateralis*+'Russian relative of *F. monguillonii*' were found in two clades: *Fissidens monguillonii+F. lateralis+F. rivularis* (PP=0.94)*,* while the clade with four specimens of the 'Russian relative of *F. monguillonii*' received maximal support, PP=1.

Such phylogenetic inferences support our preliminary observation that this 'Russian relative of *F. monguillonii*' is a good species, and as we failed to find a proper name for it, it is described here as a new species.

Taxonomy

Fissidens mosquensis Ignatov & Ignatova, species nova. Type: Russia, Moscow, Krylatskie Hills, S-faced slope below the Church of the Nativity of the Theotokos, forb meadow, on patches of bare soil among grasses, 55.755030°N, 37.423668°E, 180 m alt. 4 Apr 2023. Coll. M. Ignatov & E. Ignatova #23-1. Holotype: MHA9135059!, isotypes: LE!, MW9092494!.

Description: *Plants* small, 2–4 mm high, 0,75 mm wide including leaves, fertile plants with 5–10 pairs of leaves, growing in small patches on soil, green or slightly glaucous in shady habitats. *Stems* with weak central
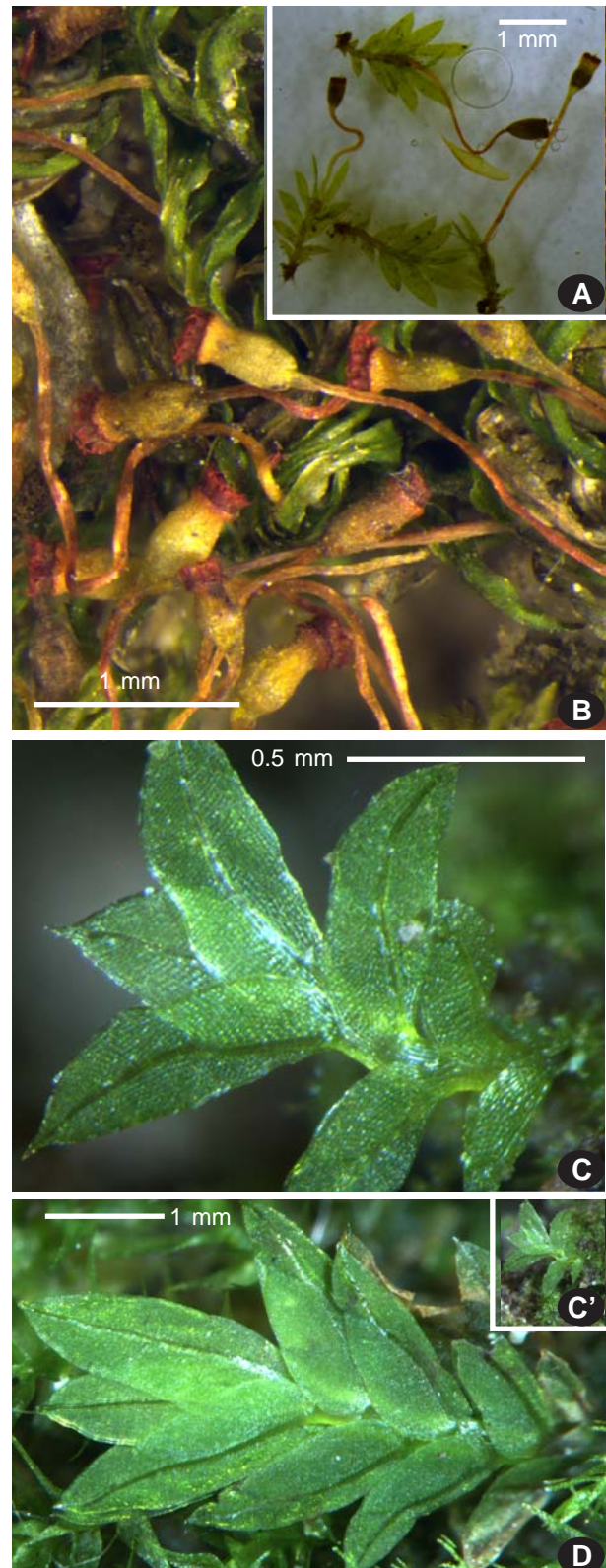
Fig. 3. A–B. *Fissidens mosquensis*, part of holotype, collected on April 4th, early-mid spring; A: dry. B: few plants in water, showing that the setae remain curved in wet state; C: young plant in type locality in mid summer; D: *Fissidens bryoides*, growing nearby the plant in C, showing contrasting size difference, as pictures at D and inset C' have the same magnification.
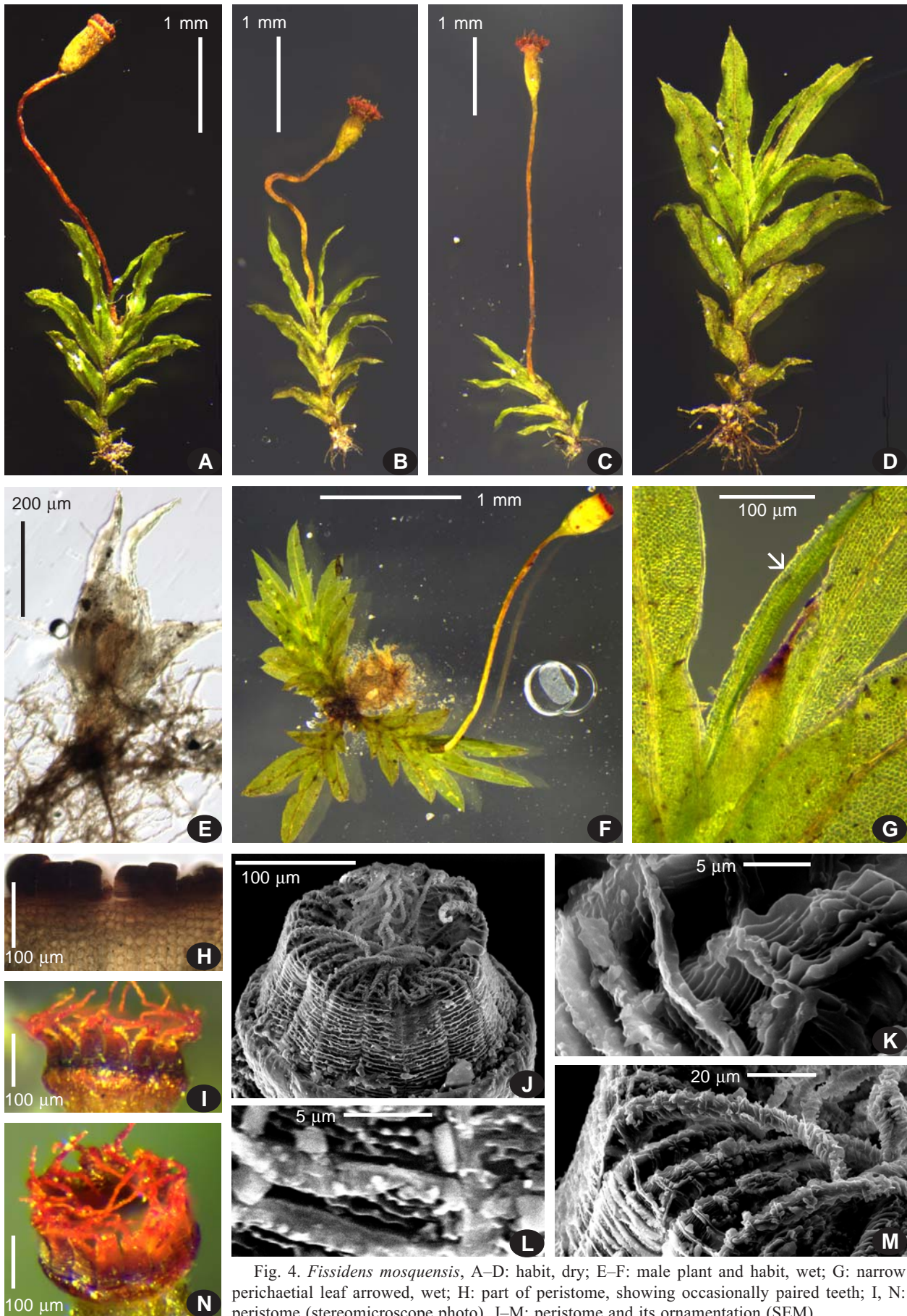
Fig. 4. *Fissidens mosquensis*, A–D: habit, dry; E–F: male plant and habit, wet; G: narrow perichaetial leaf arrowed, wet; H: part of peristome, showing occasionally paired teeth; I, N: peristome (stereomicroscope photo), J–M: peristome and its ornamentation (SEM).
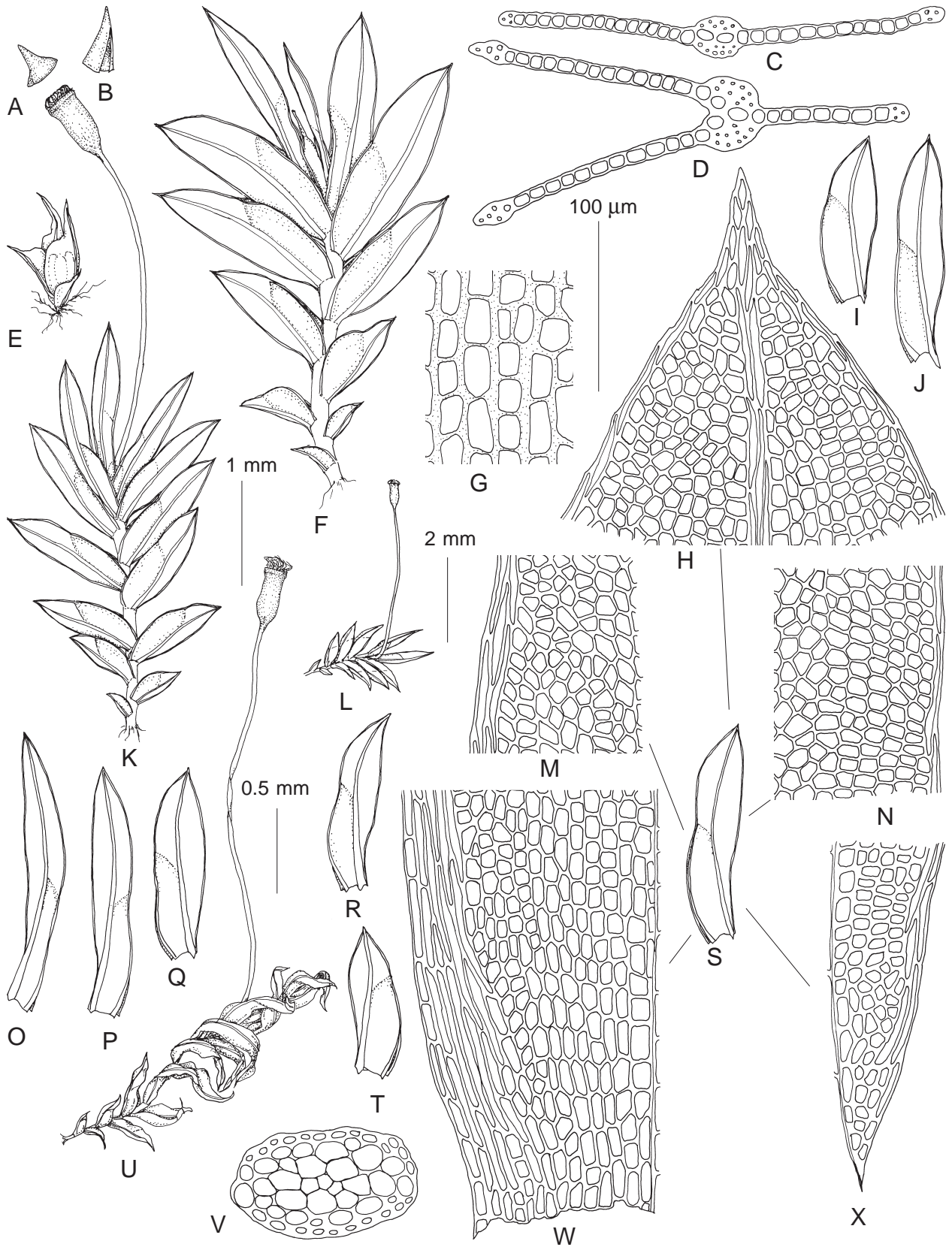
Fig. 5. *Fissidens mosquensis* (from holotype). A: operculum; B: calyptra; C–D: leaf transverse sections; E: dwarf male plant; F: plant with young sporophyte, wet; G: exothecial cells; H: upper leaf cells; I–J, Q–T: stem leaves; K–L: habit, wet; M: median cells of vaginant lamina; N: median cells of dorsal lamina; O–P: perichaetial leaves; U: habit, dry; V: stem transverse section; W: basal cells of vaginant lamina; X: lower cells of dorsal lamina. Scale bars: 2 mm for L; 1 mm for A–B, K, U; 0.5 mm for E–F, I–J, O–T; 100 μm for C–D, G–H, M–N, V–X.

Fig. 6. Habitat of the type locality of *Fissidens mosquensis*, a rather average meadow on a slope in a Moscow suburb, with small patches of bare soil among herbs, on which the species grows (arrowed). Photo in mid summer; the bare soil is more available in spring (in April, cf. Fig. 3A).

strand. *Leaves* more or less spaced, slightly flexuose when dry, erect-spreading to spreading when wet, forming 40–60° angle with stem, oblong-lanceolate, 0.7–1.3×0.25–0.3 mm, length/width ratio 3–5:1, apices acute, often apiculate; dorsal lamina narrowed below, extending to leaf insertion; vaginant laminae closed, 0.5–0.6 of leaf length; margins entire, often slightly serrulate near apex; border present on all laminae, extending to near leaf apices, not confluent with costa; cells of limbidium linear, hyaline, thick-walled, in 1–2 rows in apical and dorsal laminae, 1–2-stratose, in vaginant lamina in 2–5 rows, widened below; costae percurrent or extending into apiculus, bryoides-type; laminal cells unistratose, smooth, firm-walled, cells of apical and dorsal laminae hexagonal, with admixture of short rectangular and transversely rectangular cells, 11–15(–17)×8–12 μm, cells of vaginant laminae elongate-hexagonal and rectangular, 10–24×8–12 μm. *Sexual condition* rhizoautoicous, male plants dwarfish, on rhizoids of normal-sized female plants, antheridia ca. 150 μm long. Perichaetia terminal. *Perichaetial leaves* 1.3–1.5×0.18–0.25 mm, in perichaetia with immature sporophytes oblong-linear, much narrower and shorter than upper stem leaves. Sporophytes single per perichaetium. *Setae* 3–4 mm, curved when dry. *Capsules* erect, straight, urns 0.4–0.6×0.2–0.3 mm. Exothecial cells rectangular and quadrate, with thick longitudinal walls and thin transverse walls; stomata at urn base. *Peristome* bryoides-type, teeth 270–300 μm long. *Opercula* conic, with long beak, ca. 0.5 mm long. *Spores* 10–14 μm. *Calyptrae* cucullate, smooth.

Supplementary comments on morphology, habitat and phenology:

The species produces sporophytes in spring, i.e. similar to other species of the *F. bryoides* complex. In summer time only young plants (Fig. 3C) were encountered (Fig. 6). The habitats in the localities where we collected this species in Middle European Russia and the Urals appear unexceptional, without any special characteris-

tics. In the type locality the species grows on humus subtended by a sand of marine Cretaceous origin, slightly more calcareous compared to sands from the last glaciation. The localities in Smolensk and Kaluga Provinces were on similar slopes, with open forest or herb vegetation. The neglect of the species might result from its occurrence in such non-specific habitats, and also from the fact that in summer it lacks gametangia and capsules and therefore is difficult to identify as different from *F. bryoides*. However, when looking for morphological distinctions in the light of the molecular results, it is easy to see that *F. mosquensis* has slightly arching leaves, which helps to identify even very young individuals, as, e.g., in Fig. 3C, while *F. bryoides* has straight leaves pointing upwards (Fig. 3D).

Differentiation of *F. mosquensis*:

The most relevant comparisons are firstly with *F. monguillonii* and *F. lateralis*, which are related to *F. mosquensis* according to the molecular phylogenetic data, and secondly with *F. bryoides* s.l. and especially *F. viridulus*, the latter having a similar size and sexual condition.

The distinction from *F. monguillonii* is obvious when the plants are well-developed. The specimens from France used in our study, including one from the type locality (Fig. 7), consist of much larger plants (10–15 mm vs. 2–4 mm high); with larger stem leaves (1.7–2.8×0.6–0.7 mm vs. 0.8–0.9×0.25–0.3 mm); longer and more strongly differentiated perichaetial leaves (3.6–3.7×0.3 mm vs. 1.4–1.6×0.2–0.3 mm); and limbidium cells in 3–4 rows, 2–3-stratose vs. in 1–2 rows, 1–2-stratose.

In addition, plants of *F. mosquensis* are slightly glaucous in color, especially when young, being similar to *F. bryoides* and *F. viridulus*, whereas *F. monguillonii* is brownish, and this color was noted by Bruggeman-Nannenga (1985) as diagnostic for this species. Also, the slightly arching leaves forming a wide angle with the stem have a distinctive aspect, almost unique among sympatric *Fissidens* species. In *F. monguillonii* the leaves
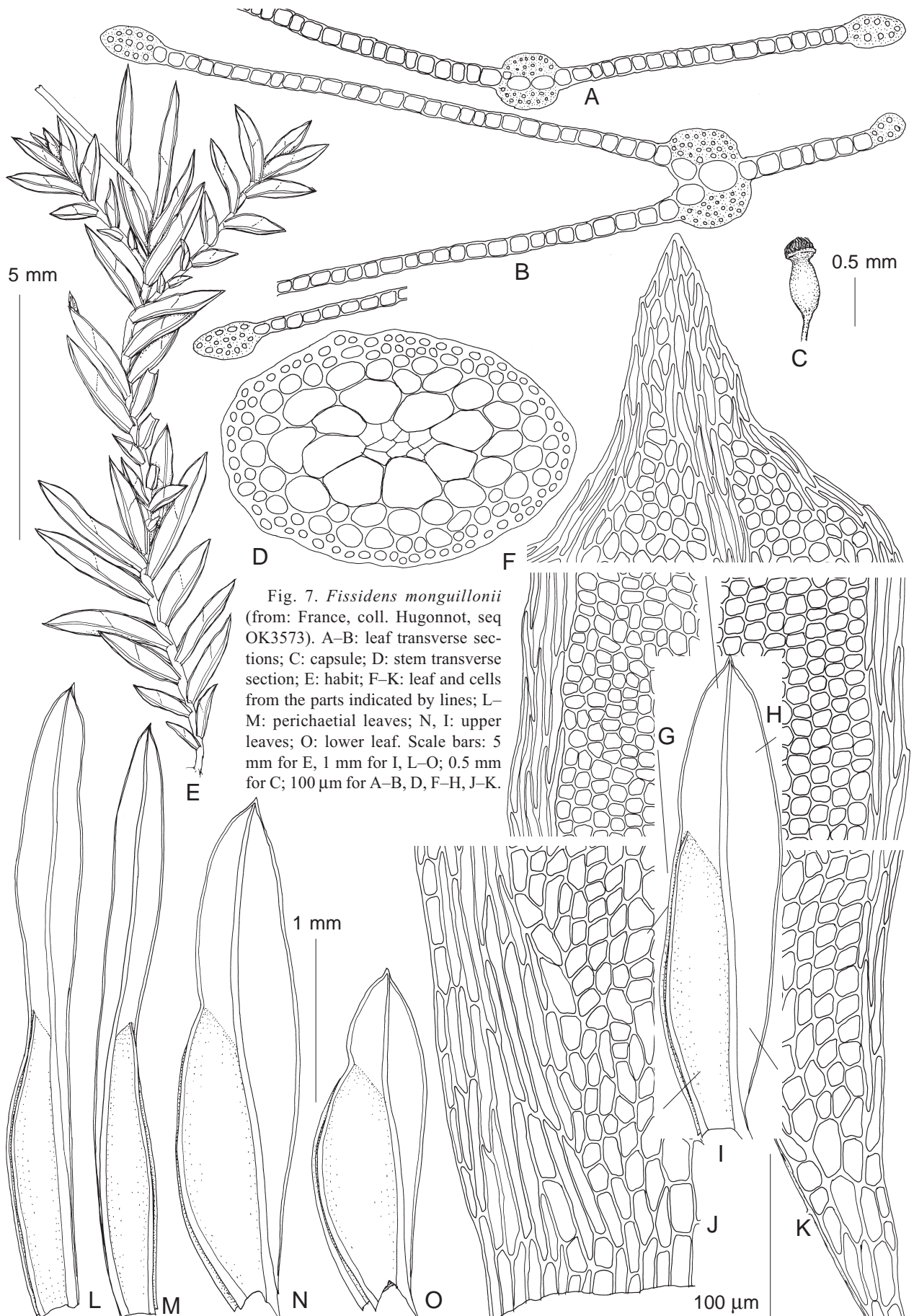
Fig. 7. *Fissidens monguillonii* (from: France, coll. Hugonnot, seq OK3573). A–B: leaf transverse sections; C: capsule; D: stem transverse section; E: habit; F–K: leaf and cells from the parts indicated by lines; L–M: perichaetial leaves; N, I: upper leaves; O: lower leaf. Scale bars: 5 mm for E, 1 mm for I, L–O; 0.5 mm for C; 100 μm for A–B, D, F–H, J–K.
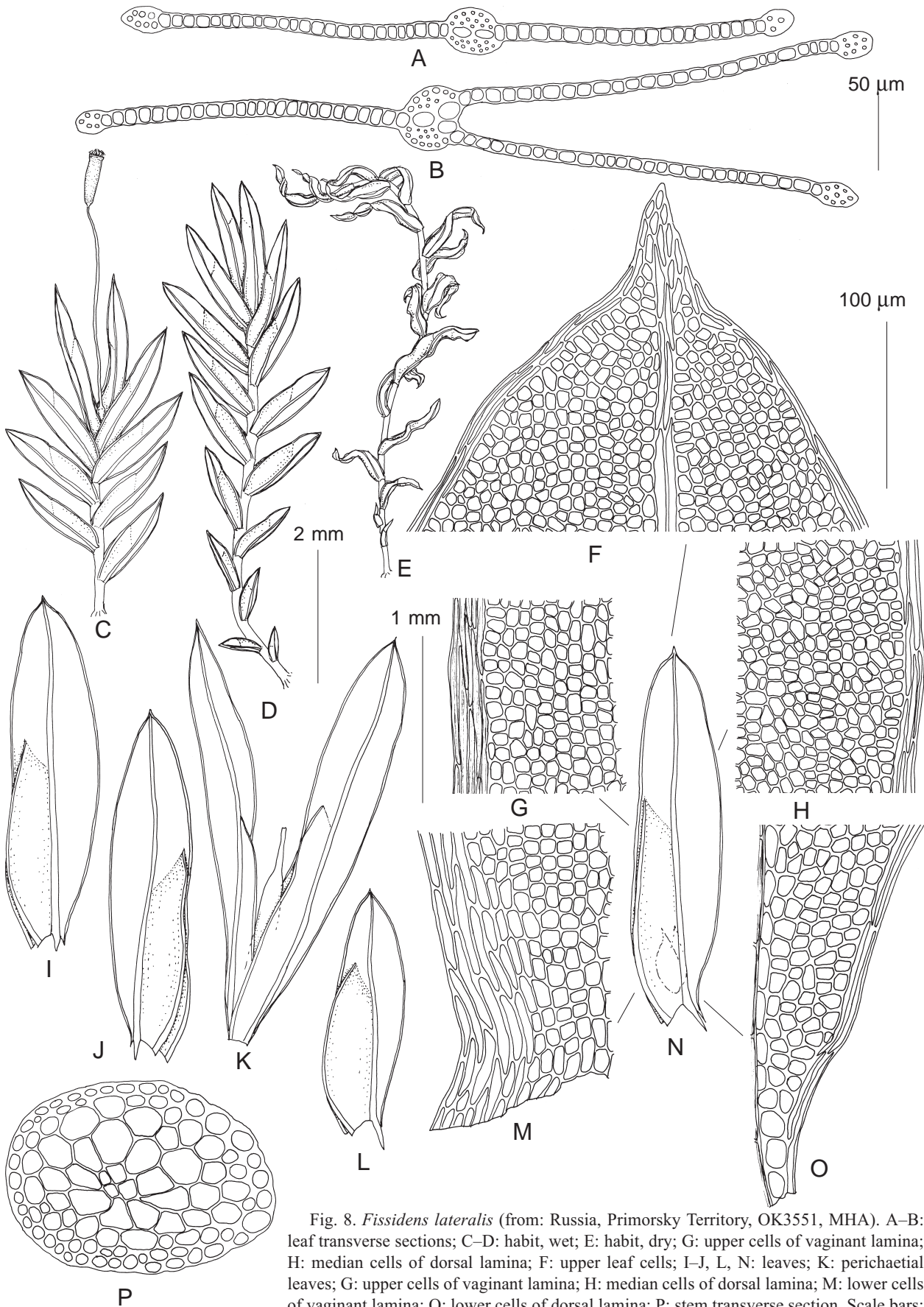
Fig. 8. *Fissidens lateralis* (from: Russia, Primorsky Territory, OK3551, MHA). A–B: leaf transverse sections; C–D: habit, wet; E: habit, dry; G: upper cells of vaginant lamina; H: median cells of dorsal lamina; F: upper leaf cells; I–J, L, N: leaves; K: perichaetial leaves; G: upper cells of vaginant lamina; H: median cells of dorsal lamina; M: lower cells of vaginant lamina; O: lower cells of dorsal lamina; P: stem transverse section. Scale bars: 2 mm for C–E; 1 mm for I–L, N; 100 μm for F, G–H, M, O; 50 μm for A–B, P.

are straight and form a rather narrower angle with the stem (45–50°). Bruggeman-Nannenga (1985) mentioned that poorly developed plants of *F. monguillonii* can be similar to *F. viridulus*. Their distinctions obviously need further studies.

Bruggeman-Nannenga (1985) also suggested a close relationship of *F. monguillonii* with *F. lateralis* Broth. (*F. bryoides* Hedw. var. *lateralis* (Broth.) Z. Iwats. & T. Suzuki). The present study tentatively supports this statement: at least a collection from the Russian Far East which fits the description of this taxon was resolved in the clade with *F. monguillonii* and *F. mosquensis* in both ITS and *trn*S-F analyses.

Fissidens lateralis (Fig. 8) is smaller than *F. monguillonii*, thus approaching *F. mosquensis* in size, but its leaves are not arching, larger than in *F. mosquensis* (1.3–1.9×0.4–0.5 mm vs. 0.7–1.3×0.25–0.3 mm) and brownish rather than glaucous. Also *F. lateralis* is usually synoicous whereas *F. mosquensis* is rhizoautoicous.

At present we know of only one specimen of *F. lateralis* from Russia: "Primorsky Territory, Ussuri Reserve, Komarovo-Zapovednoe Field Station, Komarovka River bank near valley slope, 43.64185N, 132.34560E, 152 m alt., mixed forest, on eroded mineral ground, 14.VIII.2022 Fedosov, Shkurko & Ischenko s.n." (MW). Its sequences are remarkably different from those of *F. mosquensis*, which is rather homogeneous in four studied samples.

<center>*   *   *</center>

Comparison of *Fissidens mosquensis* with species of *F. bryoides* s.l.

This is relevant because even *F. monguillonii* can be confused with *F. viridulus* (Bruggeman-Nannenga, 1985). As already indicated, the latter species name encompasses a diversity that is still not fully understood. Therefore, we describe here the distinctions between *F. mosquensis* and representatives of the clades in the molecular phylogenetic tree rather than between taxonomically defined entities. Fig. 9 shows two leaf apices for each 'species / clade representative' primarily with regard to cell areolation and the termination of the costae and limbidia; Fig. 10 demonstrates differences in leaf shape and size between these 'species / clade representatives'.

The pictures in Fig. 9 were taken from herbarium collections, using the same microscope with the same illumination. Photographs of the distal parts of two leaves from one shoot are presented. We used leaves immediately below the perichaetial leaves from maximally developed plants, on stems terminated by archegonia. To obtain depth of focus throughout the image, we took 20–30 individual photos and Z-stacked them in Helicon softrware. The latter program has three algorithms of Z-stacking; the most useful for bryophyte research, according to our experience, is method 'B'. However, cell outlines obtained using method 'B' in most studies of *Fissidens* species were not clear in parts of the image (cf. Fig. 9O). Therefore, method 'C' was applied to most of the samples. The difference from method 'B' can be seen by comparison of the two methods (Figs. 9O and 9P respectively). However, photographs of *F. mosquensis* (Fig. 9I–J) and *F. viridulus* 'caucasicus' are presented here using method 'B' Z-stacks, as method 'C' gave inappropriate images for them, with blurred cell walls. Therefore, the more conspicuously pellucid cell walls in *F. mosquensis* (Fig. 9I–J) and *F. viridulus* 'caucasicus' are, in part, better contrasted by method 'B', whereas for other species method 'B' resulted in images like those shown in Fig. 9O.

Comparisons of *F. mosquensis* with 'species / clade representatives' are given below. Not discussed here are (1) species with a contrastingly different morphology, lacking a limbidium on most parts of the leaves (as in *F. arnoldii*), (2) small epilithic species (*F. pusillus*), and (3) large aquatic mosses with a multistratose border (*F. rivularis, F. crassipes*).

Fissidens bryoides (Fig. 9A–B) is similar to *F. mosquensis* (Fig. 9I–J) in having leaves with apiculate apices and costae extending into the apiculus, but differs from it in having the leaf border confluent with the costa (vs. not confluent) and a gonioautoicous sexual condition, i.e. having bud-like perigonia situated in axils of the stem leaves, whereas in *F. mosquensis* dwarf male plants develop on the rhizoids of female plants. Plants of *F. bryoides* are larger, with longer leaves (1.3–2.7 mm vs. 0.7–1.3 mm) forming a narrower angle with the stems (cf. Figs. 3D and 3C).

***Fissidens viridulus* 'sibiricus'** (Fig. 9C–D) is characterized by acute to subobtuse leaf apices with the widest angle between the margin and the costa. Samples of this clade are highly variable in their sexual condition (synoicous, with naked antheridia and bud-like perigonia in the leaf axils, as well as with dwarf male plants at the base of female ones). However, unlike *F. mosquensis*, the costa always ends a few cells below the apiculus. The border is narrow, but is present in most stem leaves; it is never confluent with the costa

.

Fissidens gymnandrus (Fig. 9E–F) is usually readily distinguished from other similar species, including *F. mosquensis,* by having naked antheridia in axils of the stem leaves, often just one antheridium per each or almost each axil. It has leaves with the costa subpercurrent or ending a few cells below the apiculus, and the limbidium also ends shortly below the apex. In these characters this species is intermediate between *F. bryoides* and *F. viridulus* 'sibiricus'. It may also have terminal synoicous inflorescences, as observed in the type specimen of this species (Bruggeman-Nannenga, pers. comm.). *Fissidens gymnandrus* often grows on tree bases experiencing prolonged flooding.

Fissidens arcticus (Fig. 9G–H) has small leaves, with the limbidium ending more or less distant from the leaf apex, and intralaminal limbidia are usually seen in the vaginant laminae, which usually extend well above mid-leaf. It is consistently synoicous, whereas in *F. mosquensis* we observed only dwarf male plants at the base of female shoots.
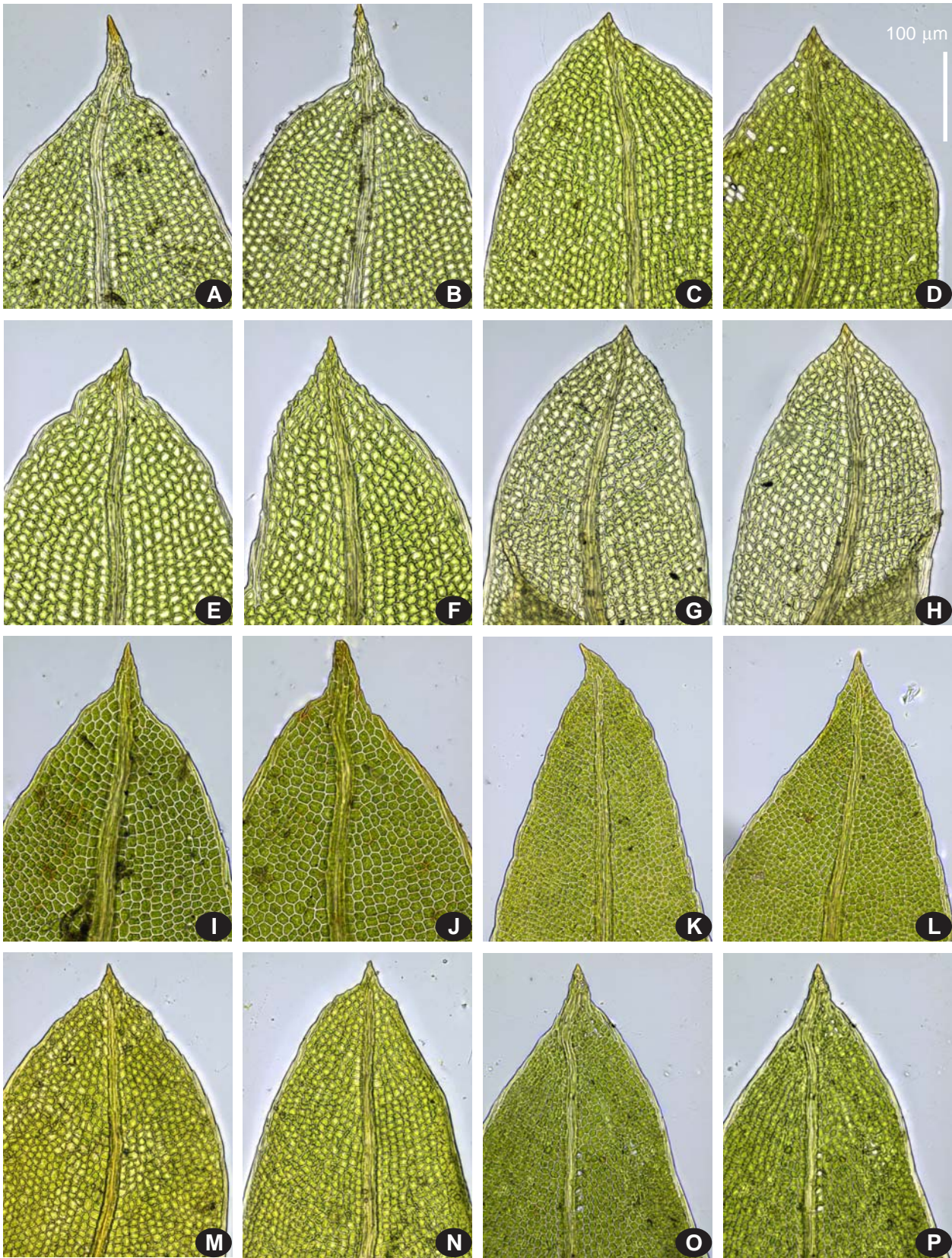
Fig. 9. Leaf acumina of *Fissidens* taxa corresponding to clades in the molecular phylogenetic tree (Fig. 1): A–B: *F. bryoides* OK344; C–D: *F. viridulus* 'sibiricus' OK352; E–F: *F. gymnandrus* OK331; G–H: *F. arcticus* OK354; I–J: *F. mosquensis* OK3555; K–L: *F. viridulus* 'caucasicus' OK498; M–N: *F. viridulus* 'orientalis' OK904; O–P: *F. viridulus* 'kamchaticus' OK481. Note that two photos of the latter species were composed from the same images using two different stacking methods, O: method 'B', and P: method 'C' in Helicon software. Images of the other species were composed using 'C', except I–L, composed using 'B'. Scale bar: 100 µm for all images.
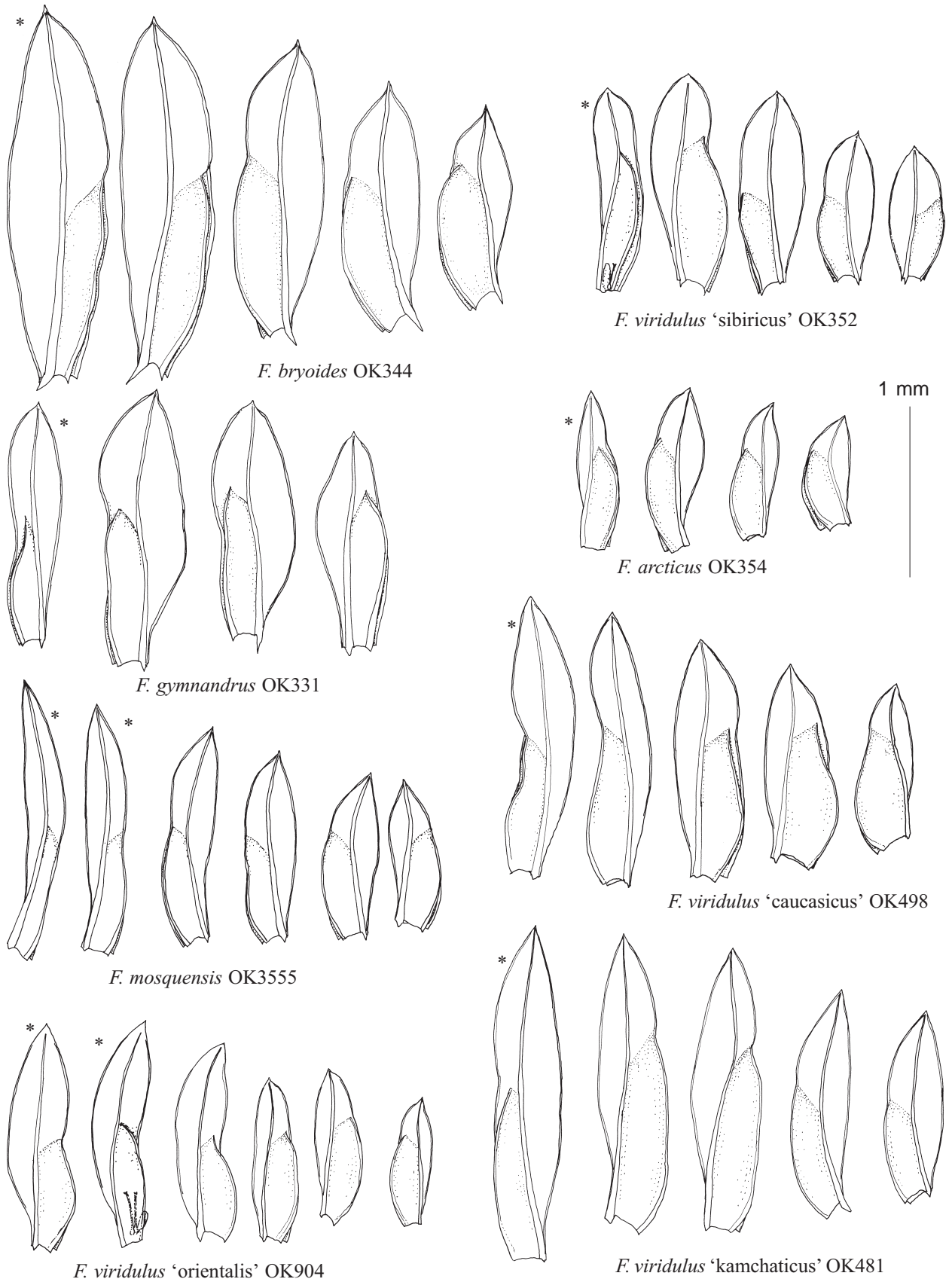
Fig. 10. Stem and perichaetial (marked with asterisk) leaves of *Fissidens* corresponding to clades in Fig. 1. Scale bar: 1 mm for all.

Fissidens viridulus '**caucasicus**' (Fig. 9K–L) is similar to *F. mosquensis* in its pellucid and apparently firm cell walls. Its upper leaf cells are not arranged in rows, which are more or less apparent in most species. The leaves are gradually tapered to the apex in the form of a narrow triangle, and this pattern is rather regular, differentiating plants of this clade from 'other viridulus' occurring in Russia. We failed to find any perigonia or dwarf male plants in a limited number of studied plants from this clade. We presume that they are likely to be rhizoautoicous, and probably can be found with antheridia in seasons other than summer, the latter being, unfortunately, the only period when field work is possible for most Russian bryologists because of teaching duties.

Fissidens viridulus '**orientalis**' (Fig. 9M–N) differs from *F. mosquensis* is its smaller leaves (cf. Fig. 10), shorter, subpercurrent costa, and the absence of a limbidium in the lower part of the dorsal lamina and sometimes in many other parts of the lower leaves. Such plants are very variable in sexual condition, which is often synoicous, but sometimes gonioautoucous.

Fissidens viridulus '**kamchaticus**' (Fig. 9O–P) has large leaves, up to 2.5 mm long, but, unlike *F. mosquensis, its* leaves are not somewhat rigid but conspicuously soft, sometimes with broadly incurved margins. The very small cells within such large leaves give the plants an unusual appearance, allowing easy recognition. Like other entities in '*F. viridulus* s.l.', the sexual condition of *F. viridulus* 'kamchaticus' is quite variable, often synoicous, sometimes with one antheridium and one archegonium in one axil, and with this pattern repeated in several leaf axils.

There is one further species described from East Europe and reported from European Russia: *F. marginatulus* Meln.; among its stated morphological distinctions are large leaf cells, suggesting a similarity with *F. mosquensis*. Differences between *F. mosquensis* and *F. marginatulus* include a 1–2-stratose vs. always unistratose leaf border (underlined as a most important distinction in the original description) and straight vs. curved capsules.

A note on Fissidens rivularis

When selecting specimens for sequencing, we encountered several collections named *F. bryoides*, which were found to be nested in the *F. rivularis* clade in the molecular analysis. Their laminal areolation closely resembled that of large aquatic forms of *F. rivularis* (cf. Fig. 11 and 12), but they have a uni- or occasionally bistratose limbidium, and the habitat was on soil on slopes in beech forest. The locality was isolated from the stream in the ravine bottom by at least several tens of meters.

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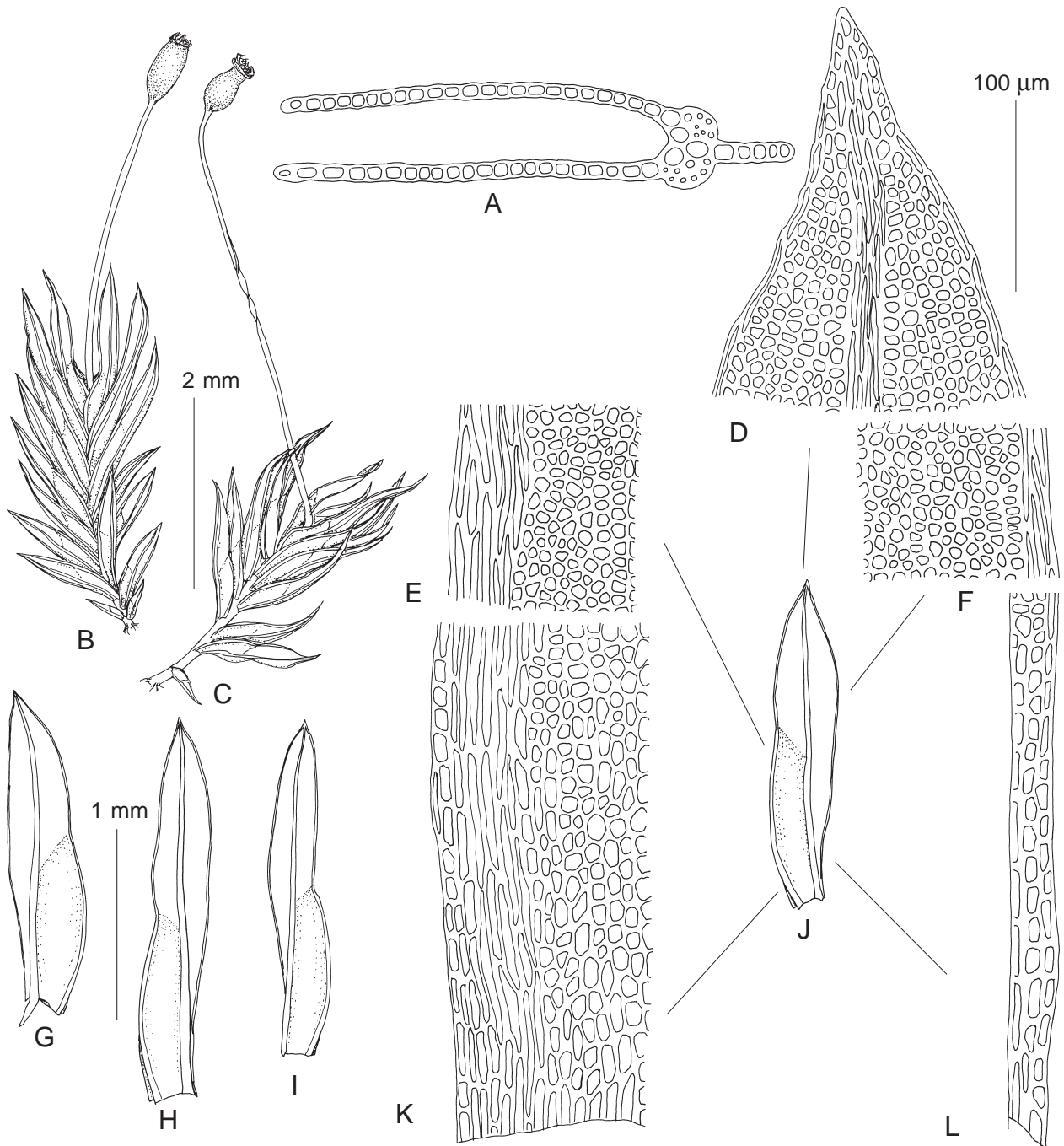
Fig. 11. *Fissidens* cf. *rivularis* (from: Russia, Krasnodar Terr., Volkonka, OK495, MHA). A: leaf transverse section; B: habit, wet; C: habit, dry; D: upper leaf cells; E: upper cells of vaginant lamina; F: median cells of dorsal lamina; G–J: leaves; ; K: lower cells of vaginant lamina; L: lower cells of dorsal lamina. Scale bars: 2 mm for B–C; 1 mm for G–J; 100 μm for A, D–F, K–L.

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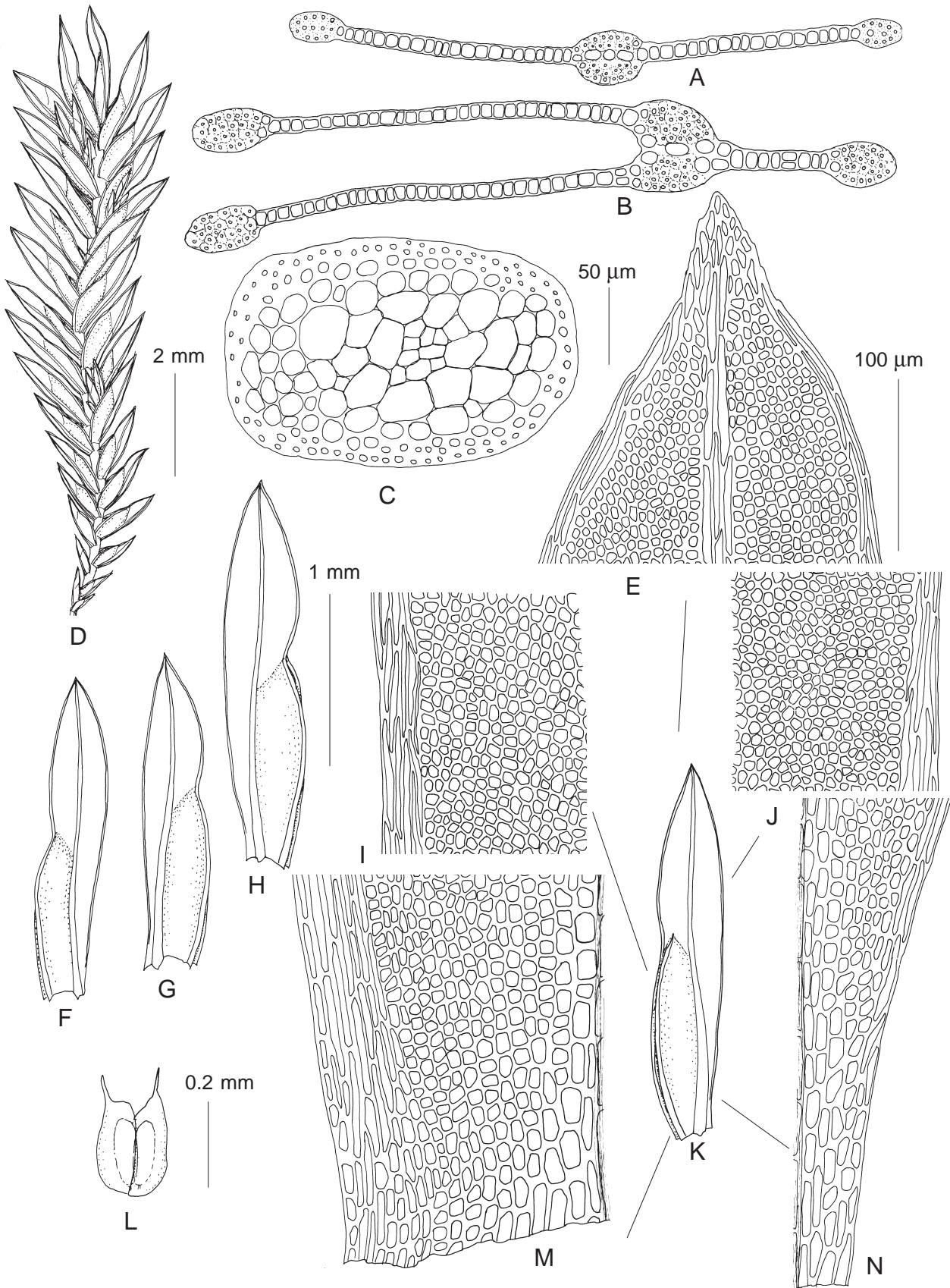
Fig. 1. *Fissidens rivularis* (from: Adjaria, Zündorf 26174, MHA). A, B: leaf transverse sections; C: stem transverse section; D: habit, wet; E: upper leaf cells; F–H K: leaves; I: upper cells of vaginant lamina; J: median cells of dorsal lamina; L: perigonium; M: lower cells of vaginant lamina; N: lower cells of dorsal lamina. Scale bars: 2 mm for D; 1 mm for F–H, K; 0.2 mm for L; 100 μm for E, I–J, M–N; 50 μm for A–C.

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SM: Appendix. Specimens of the genus *Fissidens* used for DNA studies of GenBank accession numbers: https://kmkjournals.com/upload/PDF/Arctoa/32/Arctoa_32_059_074_SM.pdf