THE GENUS *FISSIDENS* IN RUSSIA, III: *FISSIDENS KIUSIUENSIS* (BRYOPHYTA), NEW SPECIES FOR THE RUSSIAN FAR EAST

РОД *FISSIDENS* В РОССИИ, III: *FISSIDENS KIUSIUENSIS* (BRYOPHYTA), НОВЫЙ ВИД ДЛЯ РОССИЙСКОГО ДАЛЬНЕГО ВОСТОКА

VLADIMIR E. FEDOSOV^{1,2}, YU-MEI WEI³, BOON-CHUAN HO⁴, JOHN ATWOOD⁵, OXANA I. KUZNETSOVA⁶ & MICHAEL S. IGNATOV^{1,6}

Владимир Э. Федосов^{1,2}, Ю-Мэй Вэй³, Бон-Чуан Хо⁴, Джон Этвуд⁵, Оксана И. Кузнецова⁶, Михаил С. Игнатов^{1,6}

Abstract

Among recent collections of mosses from the Ussurijsky Nature Reserve (Primorsky Territory, Russia) we found a very small *Fissidens* species, which did not match any species description in its combination of morphological traits. The species resembles the North American *F. closteri* subsp. *closteri* in the mostly subentire leaf margins and having only a few stereids in the costa, but in its scabrous calyptrae it also matches the East Asian *F. closteri* subsp. *kiusiuensis*. Molecular phylogenetic study found that the nuclear ITS of these plants is subidentical with Chinese plants from Guangxi Province, and strongly different from the North American plants. Morphological variation in Asian populations is found to be broader than previously described. Therefore we suggest resurrection of *Fissidens kiusiuensis* as a separate species. The finding in Guangxi Province is the third record of this species in China. A correction is made regarding the citation of lectotype specimen of *F. closteri* in the NY herbarium.

Резюме

В Уссурийском природном заповеднике (Приморский край) был собран образец мелкого вида рода *Fissidens*, комбинация признаков которого не вполне совпадала с ранее описанными видами. С одной стороны, он сходен с североамериканским *F. closteri* subsp. *closteri* по практически цельным краям листа и слабому развитию стереид в жилке, с другой – сильно мамиллозная поверхность колпачка соответствует восточноазиатскому *F. closteri* subsp. *kiusiuensis*. Изученные последовательности ДНК ядерных спейсеров ITS выявили практически полную идентичность российского образца с *F. closteri* subsp. *kiusiuensis* из южного Китая, и значительные отличия от последовательности североамериканского образца из штата Миссури. Морфологическая изменчивость азиатских популяций оказывается более широкой, чем было описано ранее. Мы предлагаем восстановить видовой статус *Fissidens kiusiuensis*. Находка вида в Гуанси-Чжуанском автономном районе – третья для Китая. Даны уточнения в отношении корректного цитирования лектотипа *F. closteri* из гербария Нью-Йоркского ботанического сада.

KEYWORS: mosses, Russia, China, new records, Fissidens closteri, taxonomy, lectotype

INTRODUCTION

Fissidens Hedw. is a genus of more than 481 accepted species (Brinda & Atwood, 2024), with major diversity in the tropics (Pursell & Bruggeman-Nannega, 2004; Budke *et al.*, 2023). In northern and temperate floras, the number of *Fissidens* species is more limited. In the Check-list of mosses of East Europe and North Asia (Ignatov *et al.*, 2006) only 20 species of this

genus were listed from the territory of Russia, which is notably less than in Europe, 34 (Hodgetts *et al.*, 2020) or North America north of Mexico, 37 (Pursell, 2007). Recent studies found several additional species in Russia, and most of them are from the southern Russian Far East: *F. hyalinus* Wilson & Hook. (Ignatov *et al.*, 2007), *F. beckettii* Mitt. (Ignatov & Suzuki, 2018), *F. lateralis* (Ignatov *et al.*, 2023) and *F. neomagofukui* and

¹ – Lomonosov Moscow State University, Faculty of Biology, Plant Ecology and Geography Dept., Leninskie Gory Str. 1–12, Moscow 119234 Russia; e-mail: misha_ignatov@list.ru. ORCID: (MI) 0000-0001-6096-6315 (VF) 0000-0002-5331-6346

² – Botanical Garden-Institute, Far Eastern Branch of the Russian Academy of Sciences, Makovskogo Street, 142, Vladivostok, 690024, Russia

³ – Guangxi Institute of Botany, Chinese Academy of Science, Guangxi Zhuang Autonomous Region, Guilin 541006 China; ORCID: 0000-0002-1845-716X;

⁴ – Herbarium, Singapore Botanic Gardens, National Parks Board, 1 Cluny Road, 259569, Republic of Singapore; ORCID: 0000-0003-0530-775X

⁵ – Missouri Botanical Garden, 4344 Shaw Blvd., St. Louis, Missouri 63110 U.S.A;ORCID: 0000-0002-8646-6711.

⁶ – Tsitsin Main Botanical Garden, Russian Academy of Sciences, Botanicheskaya Str., 4, Moscow 127276 Russia; ORCID: (OK) 0000-0002-5513-1329

F. extremiorientalis (Ignatova *et al.*, 2023). This is not especially surprising considering the great diversity of the genus in Japan: Noguchi & Iwatsuki (1987) reported 42 *Fissidens* species in the 'Illustrated moss flora of Japan', and in the 'New catalog of the mosses of Japan' 46 species and 5 varieties were listed (Iwatsuki, 2004).

In the course of a recent exploration of the Far Eastern Russian moss flora, another enigmatic *Fissidens* was collected in the Ussurijsky Reserve (Primorsky Territory). The plant is very tiny, with elimbate leaves and scabrose calyptrae, characters suggesting affinity to *F. closteri* Austin subsp. *kiusiuensis* (Sakurai) Z. Iwats. However, it has a number of differences from that taxon as reported in literature (Iwatsuki & Suzuki, 1982; Noguchi & Iwatsuki, 1987), and agrees somewhat with *F. closteri* var. *closteri*. In the latter subspecies, the leaves are subentire vs. clearly serrulate, and the costa lacks welldeveloped stereids, which are reported for Japanese plants by Iwatsuki & Suzuki (1982, Table 6, Pl. XXIV).

To resolve the identity of the Russian plants, a molecular phylogenetic study and morphological comparison with the plants from other regions were undertaken.

MATERIALS AND METHODS

Molecular studies. Nuclear ITS was selected for analysis based on previous studies, as the most informative region for DNA barcoding purposes in various moss families, including *Fissidens* (Guerra *et al.*, 2021; Ignatov *et al.*, 2023; Ignatova *et al.*, 2023). Plastid rps4 region was also used; however, as the sequences for this region in GenBank was available for different species, the nuclear and plastid data were analysed separately.

Fissidens species with limbate leaves were included in the analysis, since Suzuki *et al.* (2018) suggested the secondary loss of the limbidium in *F. exilis* Hedw., *F. pseudoclosteri* Z. Iwats. & S.S. Kumar, and *F. protonematicola* Sakurai, and moreover other species were found almost impossible to align.

PCR, sequencing

The molecular studies followed the laboratory protocol for DNA extraction, amplification and sequencing described in, e.g., Gardiner *et al.* (2005) for ITS and Hernández-Maqueda *et al.* (2008) for *rps*4.

Sequences were aligned using MAFFT v. 7.505 (2022/ Apr/10) with E-INS-i aligning strategy with otherwise default options.

Maximum likelyhood analysis was performed at W-IQ-TREE server (Trifinopoulos *et al.*, 2016), 1000 replications and otherwise default parameter. Substitution models were selected by the server: for ITS1: HKY+F+G4; 5.8S RNA gene K2P+I; ITS2: TIM2+F+G4 and for *rps*4: part of trnS gene: K2P; trnS-rps4 intergenic spacer: TPM2u+F+I; rps4 gene K3Pu+F+G4.

The ITS tree was rooted on *F. serrulatus*, the sequence of which differs strongly from most of the other studied species, but still possible to align. The rps4 tree was rooted on *F. nobilis*, a species with limbate leaves.

Morphological studies

In addition to standard microscopic observations, peristomes were studied under SEM by SNE-4500M, coated by gold. 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

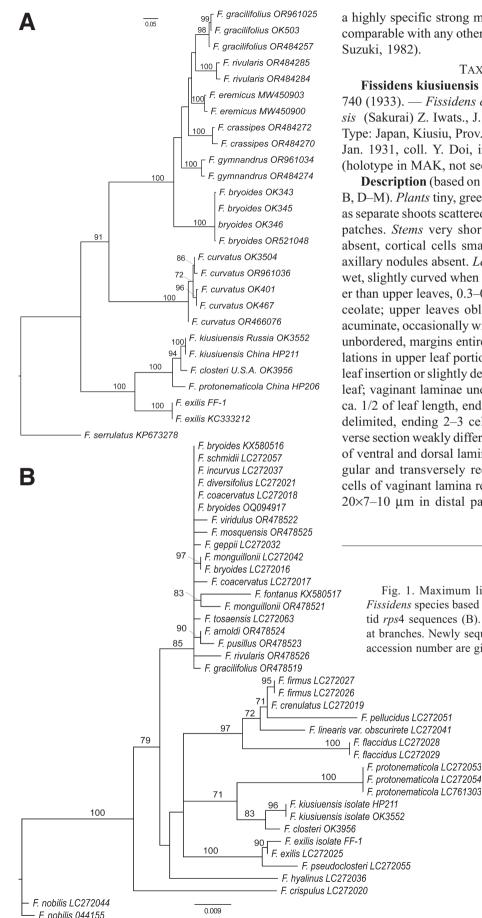
Consensus molecular phylogenetic tree of ITS from ML analysis (Fig. 1A) rooted on *Fissidens serrulatus* results in two clades. One clade includes *Fissidens* species with limbate leaves: *F. bryoides*-group and *F. curvatus* (BS=94). Another clade includes *F. exilis*, *F. protonematicola* and *F. closteri* s.1. (BS=100). In the latter clade, the clade of *F. exilis* (BS=100) splits off first, and the clade sister to it is also maximally supported and includes *F. protonematicola* and clade of *F. closteri* s.1. (BS=94). Within the latter, North American *F. closteri* var. *closteri* is sister to clade of Chinese and Russian plants that is maximally supported (Fig. 2).

From the alignment, the distinction of the plants of the *F. closteri*-complex is very clear: Chinese and Russian plants of *Fissidens closteri* subsp. *kiusiuensis* are subidentical (differ only in one indel), while the East Asian and North American plants of *F. closteri* subsp. *closteri* differ in 19 substitutions and 10 indels of different length. Another presumably related species, *F. protonematicola* is far more distinct: it differs from Chinese plants of *F. closteri* subsp. *kiusiuensis* in 36 substitutions and 15 indels of different length, from 1 to 5 nucleotides). North American *F. closteri* subsp. *closteri* and *F. protonematicola* distinctions are slightly larger, 43 substitutions and 15 indels.

Consensus molecular phylogenetic tree of *rps*4 from ML analysis rooted on *Fissidens nobilis* results in a resolved topology shown in Fig. 1B. The species that are in the focus of the present study, *F. closteri* s.l., were found in the clade sister to *F. protenematicola*, similarly to ITS analysis in Fig. 1A. *Fissidens exilis* was not found in the clade with these species. Similarly to the ITS tree, the support of two Asian *F. closteri* subsp. *kiusiuensis* was notably higher (BS=96) than that for a mutual clade of Asian and American plants (BS=83), and for the combined clade of *F. closteri* s.l. and *F. protonematicola* (BS=71).

Morphological differentiation of *F. closteri* subsp. *kiusiuensis* and subsp. *closteri* are discussed under the 'Taxonomy' section; however, as it generally is consistent with the molecular distinction, we suggest resurrecting *Fissidens kiusiuensis* as a taxon of its own at the species level.

A certain hesitation due to the unsuccessful attempts to sequence Japanese specimens may be compensated by



a highly specific strong mamillosity of the calyptra, incomparable with any other Japanese species (Iwatsuki & Suzuki, 1982).

TAXONOMY

Fissidens kiusiuensis Sakurai, Bot. Mag. Tokyo 47: 740 (1933). — *Fissidens closteri* Aust. subsp. *kiusiuensis* (Sakurai) Z. Iwats., J. Jap. Bot. 33: 249 *f.* 2 (1958). Type: Japan, Kiusiu, Prov. Satsuma, Stadtchen Izüin, 27 Jan. 1931, coll. Y. Doi, in herb. K. Sakurai, no. 1681 (holotype in MAK, not seen).

Description (based on Russian plants, Figs. 2, 3, 4A-B, D-M). Plants tiny, green or yellowish-green, growing as separate shoots scattered on substrate or forming loose patches. Stems very short, ca. 0.1 mm, central strand absent, cortical cells smaller than inner cells; hyaline axillary nodules absent. Leaves in 2-3 pairs, erect when wet, slightly curved when dry; lower leaves much smaller than upper leaves, 0.3-0.6×0.10-0.15 mm, ovate-lanceolate; upper leaves oblong, 0.8-1.1×0.15-0.25 mm, acuminate, occasionally with 1-celled apiculus; all leaves unbordered, margins entire, uneven or with blunt crenulations in upper leaf portion; dorsal lamina extending to leaf insertion or slightly decurrent, 6-8 cells wide in midleaf; vaginant laminae uneven, smaller vaginant lamina ca. 1/2 of leaf length, ending on costa; costa thin, welldelimited, ending 2-3 cells below leaf apex, in transverse section weakly differentiated, lacking stereids; cells of ventral and dorsal laminae subquadrate, short rectangular and transversely rectangular, $10-20 \times 10-15 \ \mu m$; cells of vaginant lamina rectangular to rhomboidal, 12-20×7-10 µm in distal part, elongate-rectangular, 20-

Fig. 1. Maximum likelyhood phylogenetic trees of *Fissidens* species based on nrITS sequences (A) and plastid *rps4* sequences (B). Bootstrap support >70 is shown at branches. Newly sequenced specimens with GenBank accession number are given in Appendix.

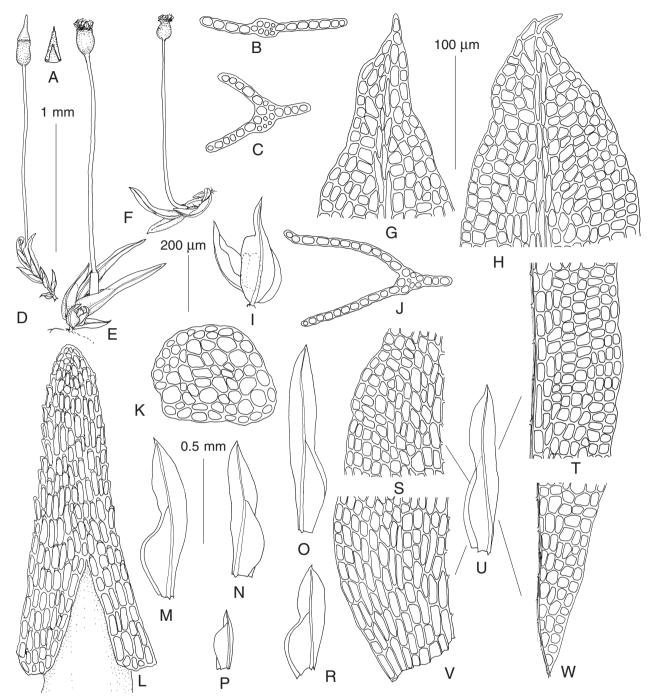


Fig. 2. *Fissidens kiusiuensis* (from Russia, Fedosov *et al.*, MHA9131858). A, L: calyptrae; B–C, J: leaf transverse sections; D: habit, dry; E–F: habit, wet; G–H: upper leaf cells; I: perigonium; K: stem transverse section; M–N, P–R, U: leaves; O: perichaetial leaf; S: upper cells of vaginant lamina; T: median cells of dorsal lamina; V: lower cells of vaginant lamina; W: lower cells of dorsal lamina. Scale bars: 1 mm for A, D–F; 1 mm for M–O, P–R, U; 200 µm for I; 100 µm for B–C, G–H, J–L, S–T, V–W.

 $35 \times 7-8$ µm in proximal part. Sexual condition rhizoautoicous; perigonia bud-like at stem bases. Perichaetia terminal. Setae straight, yellowish, 1.4–1.8 mm. Capsules erect, ovate, constricted below mouth when dry, ca. 0.2× 0.15 mm; exothecial cells subquadrate, collenchymatous, stomata few in lower part of capsule. Opercula high conic. Peristome of scariosus-type; outer surface below bifurcation smooth, with densely arranged low trabeculae, inner surface below bifurcation with spaced, high trabeculae covered with high papillae, above bifurcation without trabeculae, with spiral thickenings on both surfaces. *Spores* 9–12 μ m in diameter, looking smooth in light microscope, on SEM photos with low papillae. *Calyptrae* cucullate, ca. 0.3 mm long, scabrose due to projecting upper ends of surface cells.

Distribution and ecology

The only Russian locality of *Fissidens kiusiuensis* is in Ussurijsky Nature Reserve, where many other south-

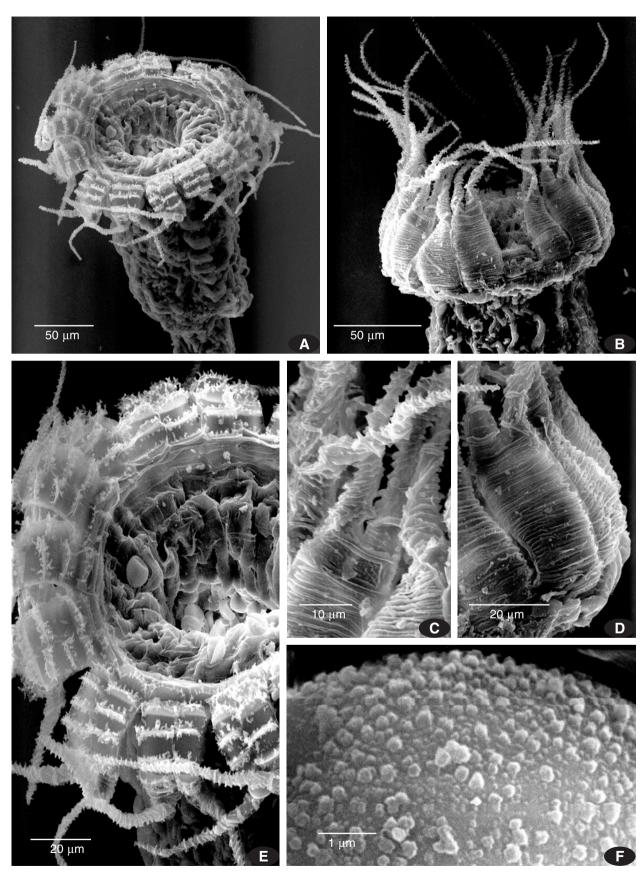


Fig. 3. *Fissidens kiusiuensis* (from Russia, *Fedosov et al.*, MHA9131858). A–B: peristome; C: outer and inner surfaces of peristome teeth above bifurcation; D: outer surface of peristome teeth below bifutcation; E: inner surface of peristome teeth; F: spore.

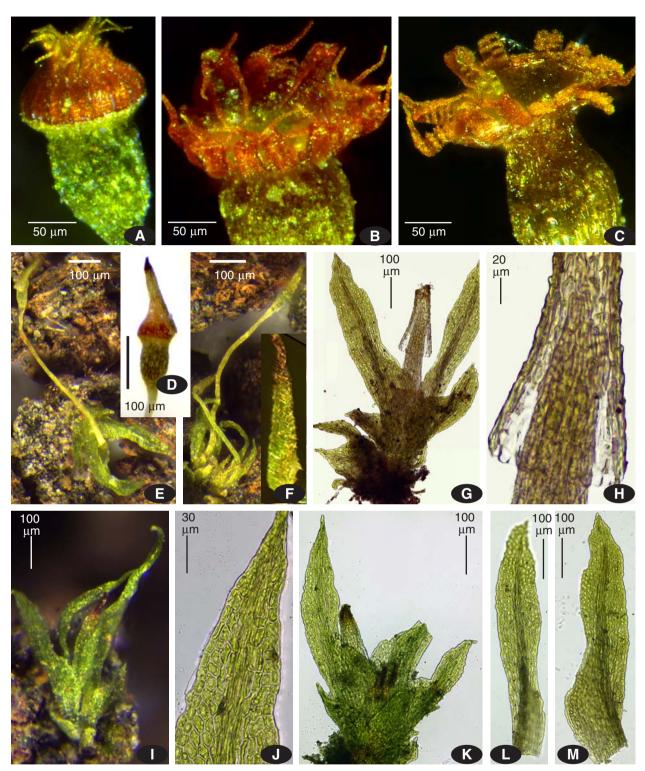


Fig. 4. *Fissidens kiusiuensis* from Russia, *Fedosov et al.*, MHA9131858 (A–B, D–M), and *F. closteri* from USA, *Atwood*, MO 3768 (C). A–C: peristomes; D: Capsule, still operculate; E–G, I, K: habit of plants with young sporophytes, still covered by calyptrae; H: calyptra with scabrous surface, close up of G; J: leaf from plant in 'I', distally attenuate; L–M: leaves, showing variation, cf. also 'J' and 'K' for the leaf margin serration.

ern East Asian species are known at their northernmost limits (Ishchenko *et al.*, 2022). *Fissidens kiusiuensis* was found growing in the mixed conifer–broad-leaved forest on a boulder along roadside:

Primorsky Territory, Ussurijsk District, Ussurijsky

Nature Reserve, upper course of Komarovka River basin, 43.64998°N, 132.46965°E, 402 m alt., the road towards the pass to Artyomovka River valley, mixed forest, on boulder near the road, 17 Aug 2022 Coll. Fedosov, Shkurko & Ischenko s.n. (MW, MHA9131858).

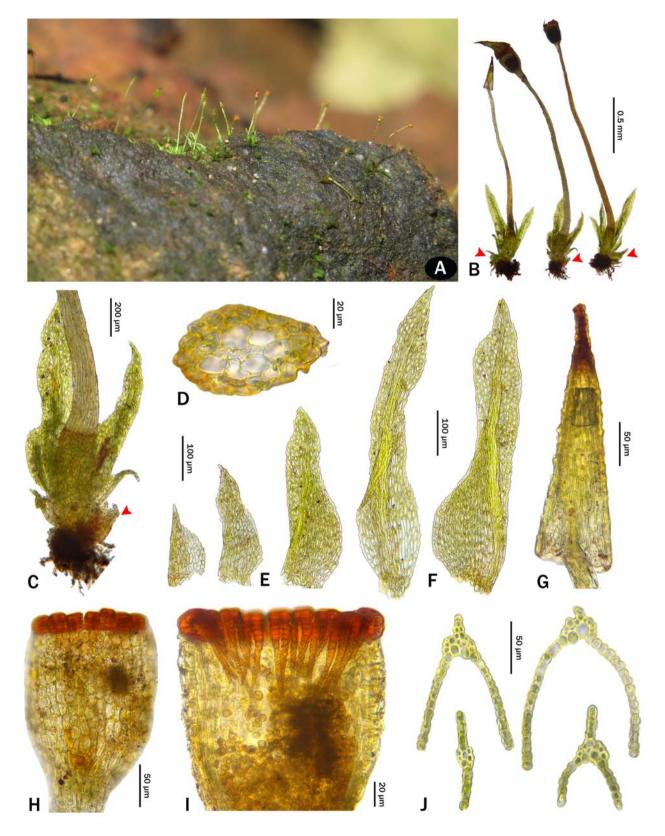


Fig. 5. *Fissidens kiusiuensis* from China, Guangxi (Ping Huang, Yu-Mei Wei, Qi-Ming Tang, Shun-Li Wang, # 4503282023BRY2309). A: habit in nature; B: habit showing gametangia distribution, male inflorescences indicated with arrows; C: close up of one of 'B'; D: stem transverse section; E: lower stem leaves; F: perichaetial leaves; G: calyptra; H: capsule, showing exothecial cells; I: capsule with reflexed peristome teeth; J: leaf transverse sections, showing solitary stereids.

In China *Fissidens kiusiuensis* was recently found in Guangxi province, while previously it was reported from Xizang (Li & Iwatsuki, 2001) and Guandong (Liu *et al.*, 2022).

China, Guangxi. Longsheng Co., Jiangdi Town, Nitang Village, Mianhuachong Tun., 25°54'7.48"N, 110°14'19.5"E, 350 m, on rock. 9 Sep 2023 Coll. Ping Huang, Yu-Mei Wei, Qi-Ming Tang, Shun-Li Wang, # 4503282023BRY2309 (IBK).

Selected Japanese collections studied:

Japan, Kyushu, Tsushima Is.,Nagasaki pref., Izahara, in a valley above Manshoin Temple, 20-220 m alt., on soil, 18 Nov 1969 Z. Iwatsuki 740 (TNS 10748), Z. Iwatsuki 760 (TNS 10786).

Comparison of Fissidens closteri and F. kiusiuensis

In addition to the main morphological distinction in the mostly smooth calyptra (mammillose only near the tip) in American plants vs. a scabrous calyptra in the Asian ones, comparisons of traits of these two species, accepted as subspecies, were published by Crum & Anderson (1981) and Iwatsuki & Suzuki (1982). The former authors noted that the costa is stronger in the Asian plants, and that the leaf margin is distinctly serrate. The latter publication summarized differences of North American vs. Asian taxa by smaller leaves (0.4-0.75 vs. 0.3-0.9 mm long), leaf margin subentire vs. serrate, shorter seta (1.2 vs. 1.3-4.0 mm), and smaller spores 7-10 vs. 10-15 µm. Iwatsuki & Suzuki (1982) did not confirm excurrent costa in Japanese plants, mentioning that by these traits the taxa are subidentical. However, the costa transverse section was found to possess many stereids, which is not the case in North American plants, nor in Asian ones observed by us.

Selected North American collections studied:

U.S.A. Missouri, Saint Genevieve County, Hickory Canyon Natural area, Benne Tract, cobble bed along small drainage leading into the south branch of Hickory Creek, 37°52'18"N 090°18'30"W, 807 ft. On top of a 6 inch piece of shaded, dry cobble, with *Hypnum cupressiforme* and *Fissidens bryoides*, 12 Oct 2019 Atwood 3768 (MO, dupl. MHA).

Iwatsuki & Suzuki (1982) mentioned that the North American plants have scabrous calyptrae, which disagrees with the descriptions in Crum & Anderson (1981) and Pursell (2007), as well as lectotype and several syntype specimens of the species (FH, NY, BM001094038!), and high-quality photomicrographs depicting Alabama, Mississippi and North Carolina specimens of *F. closteri* taken by Paul Davison at the University of North Alabama:

https://www.southernappalachianbryophytes.org/ fissidensclosteri.html

Fissidens kiusiuensis is indeed a variable species. The shorter leaves are roughly crenulate (Figs. 4K, 5E), whereas some longer ones are attenuate (Figs. 4J, 5F), and then in the distal half have the margin subentire. A similarly subentire upper leaf margin has been mentioned for this species recently by Kwon (2021) for Korean plants.

Correction of the lectotype of Fissidens closteri

Fissidens closteri Austin, Bull. Torrey Bot. Club 5(5): 21. 1874. Lectotype. [U.S.A., New Jersey] Nagel's Ravine, Sept-4-1871, *Austin* (NY (NY00324944!), designated by Iwatsuki 1958:25 as "U.S.A.; New Jersey—near Closter, 1865, *Austin*—holotype"); isolectotype. FH (FH01138426!).

C.F. Austin's (1874) protologue for *F. closteri* has the type citation: "On stones along woodland rivulets, on the Palisades, near Closter, New Jersey", as well as references to W. S. Sullivant's (1875) *Icones Muscorum, Supplement*. Seven specimens of original material are preserved in Austin's personal herbarium, now at NY, in addition to three specimens of original material in Sullivant's personal herbarium, now at FH. All of these specimens belong to *F. closteri*, include notes or labels in Austin's handwriting and span approximately eight-years of collecting dates from 1865 to 1872.

Iwatsuki (1958) designated the oldest among these as the lectotype, citing: "U.S.A.; New Jersey-near Closter, 1865, Austin-holotype (NY)", although his annotation slip does not appear on any of the NY specimen packets. One specimen packet in NY from Austin's herbarium (NY00324944!) has the label "Nagel's Brook, Sept. 1865" and "Anderson's Brook, Aug. 12th 1872" and is the best match for Iwatsuki's type citation due to the inclusion of "1865" on the label. The packet contains several slips of paper with notes in Austin's handwriting. On one of these is "Sept. 9 1865, with Jung. pumila", and below that is "Seligeria pusilla" crossed out with "F. closteri Aust." written beneath it and "sent all to Sulliv!". Also in that same NY packet is a piece of paper with four clumps of rock glued to it with the note "Nagel's Ravine, Sept-4-1871". Since the 1865 material has long been absent from the NY packet, this specimen would have been the only material available to Iwatsuki from this packet in 1958.

In FH is a specimen packet (FH01138425!) bearing Sullivant's stamp with the label "Fissidens closteri n.sp. (with Jung. pumila With.) on earth along a rivulet, 1 mi. N. East of Closter Station, July & Sept. 1865, CFA." It contains a few plants of *F. closteri* and a note "See good specimen, Rec'd Sept. 7 1871." This note references a much larger specimen (FH01138426!) that was used by Sullivant for his illustration of *F. closteri* (1875, Pl. 29, PL. 9A) according to notes on the packet and copy of the illustration.

Since there is no specimen of *F. closteri* in NY from 1865, Art. 9.19 of the IUCN (Turland *et al.* 2018) does not apply. Either the date from Iwatsuki's (1958) type citation must be corrected to 1871 since that is the date on the specimen, or the institution that he cited must be corrected to FH since that is where the 1865 material is deposited. Alternatively, a new lectotype could be designated from among the original material, although that would undesirably change the date of typification. Rath-

er, we prefer to correct the date cited by Iwatsuki for the NY specimen to 1871. Doing so preservers the NY packet that Iwatsuki considered to be 'holotype' material and the plants thought to be from 1865. The 1865 specimen in FH is small and incomplete compared to the 1871 specimen at NY that seemingly was used by Austin for his description of *F. closteri*. The FH duplicate of the 1871 specimen was furthermore used by Sullivant for his illustration of the species.

New Jersey, [Bergen County], 1 mile north east of Closter Station, July and Sept. 1865, *C.F. Austin* (FH01138425); one half mile n.e. of Closter, 4 Sept. 1871, *C.F. Austin*, (FH01138426); on stones, bank of rivulets on the palisades near Closter, 8 Sept. 1871, *C.F. Austin* (FH01138427); Anderson Brook, 12 Aug. 1872, *C.F. Austin* (NY00324945); near Closter, Sept. 1872, *C.F. Austin* (NY00304571); Closer, Aug. 12th 1872, *C.F. Austin* (NY00304573); Closer, Aug. 12th 1872, *C.F. Austin* (NY00304573); *Austin 479*, *Musci Appalachiani*, on rocks along rivulets, palisades, near Closter (Aug., Sept.) (NY00304574, NY00304575).

ACKNOWLEDGEMENTS

We are grateful to Yuya Inoue for arranging loan of Japanese specimens from TNS, to Elena Ignatova for line drawing for this paper, to Maria Alida Bruggeman-Nannenga for helpful criticism of the manuscript, and to John Brinda for the clarification of nomenclatural issues. Study was supported by Institutional project 122042700002-6 for MI, and 122042500074-5 for OK, and by RSF project 23-14-00043 for VF. The collecting of Chinese materials was supported by the National Natural Science Foundation of China (no. 32360056 for Y.M. Wei).

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Species	Isolate	Locality	Voucher	ITS	rps4
F. closteri	OK3956	USA, Missouri	Atwood 3768 (MO, dupl. MHA)	PQ737534	PQ731901
F. kiusiuensis	OK3552	Russia, Primorsky	Fedosov, Shkurko & Ischenko s.n. (MHA9131858)	PQ737535	PQ731903
F. kiusiuensis	HP211	China, Guangxi	Ping Huang, Yu-Mei Wei, Qi-Ming Tang,		
			Shun-Li Wang, # 4503282023BRY2309 (IBK)	PQ737536	PQ731902
F. protonematicola	HP206	China, Guangxi	Yu-Mei Wei, Qi-Ming Tang, Ping Huang,		
			# 4503282023BRY1759 (IBK)	PQ737537	
F. exilis	FF-1	Russia, Moscow	Fedosov (MW9075219)	PQ737538	PQ731904
F. bryoides	OK343	Russia, Moscow	Ignatov, 11 Jun 1986, s.n. (MHA9062893)	PQ737539	
F. bryoides	OK345	Russia, Moscow	Ignatov, 15 Apr 1984, s.n. (MHA9062891)	PQ737540	
F. bryoides	OK346	Russia, Moscow	Ignatov, 10 May 1986, s.n. (MHA9062890)	PQ737541	
F. curvatus	OK467	Russia, Amur Prov.	Bezgodov 14 Jul 2010 #419 (MHA)	PQ737542	
F. curvatus	OK401	Russia, Primorsky	Malashkina, 22 Sep 2001, (VBGI)	PQ737543	
F. curvatus	OK3504	Russia, Primorsky	Kolesnikova 19-307 MHA	PQ737544	
F. gracilifolius	OK503	Russia, Adygeia	Ignatov, 30 Aug 1999, s.n. (MHA)	PQ737545	

Appendix. Species of the genus Fissidens sequenced de novo for nrITS and plastid rps4.

TURLAND, N.J., J.H. WIERSEMA, F.R. BARRIE, W. GREUTER, D.L. HAWKSWORTH, P.S. HERENDEEN, S. KNAPP, W.-H. KUSBER, D.-Z. LI, K. MARHOLD, T.W. MAY, J. MCNEILL, A.M. MONRO, J. PRADO, M.J. PRICE, & G.F. SMITH (eds.) 2018: International Code of Nomenclature for algae, fungi, and plants (Shenzhen Code) adopted by the Nineteenth International Botanical Congress Shenzhen, China, July 2017. *Regnum Vegetabile* **159**. *i*–xxxviii, *1*–254. DOI: 10.12705/ Code.2018.

Received 9 October2024

Accepted 3 December 2024