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## A new species of *Stemonitis pseudoflavogenita* from Russia, and the first record of *Stemonitis capillitionodosa* in Eurasia

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### Abstract

This paper reports a new *Stemonitidaceae* species, *Stemonitis pseudoflavogenita*, found in Krasnodar Territory and Novosibirsk Region of Russia. We provide morphological and molecular characterization of *S. pseudoflavogenita* and data on its distribution and habitat. Compared to other representatives of the genus *Stemonitis*, *S. pseudoflavogenita* and three other species of this genus (*S. capillitionodosa*, *S. flavogenita*, and *S. sichuanensis*) have thickening and widening of the column apex, which makes them a separate morphological group. However, the above three known species have warted and spinulose spore ornamentations, *S. pseudoflavogenita* shows complex ornamentation consisting of large warts and a few small warts irregularly scattered among the large ones and small indentations on the spore surface. *Stemonitis capillitionodosa* is a rare myxomycete. We found and retrieved the specimens of *Stemonitis capillitionodosa* from the Laplandskiy Biosphere Reserve (Murmansk Region, Russia), the first record of this species in Eurasia. We present a scanning electron microscopic study of both *S. pseudoflavogenita* and *S. capillitionodosa*. New sequences for SSU rDNA of *S. pseudoflavogenita* and *S. capillitionodosa* were obtained.

**Keywords:** molecular data, morphology, myxomycetes, SEM, SSU rDNA gene sequences, Stemonitidales, taxonomy

### Introduction

*Stemonitidaceae* is a large family of Myxomycetes. Since Elias Magnus Fries established this family in 1829, 19 genera and 239 species have been reported worldwide (Lado 2005–2019). *Stemonitis*, a common genus of *Stemonitidaceae*, was proposed by Gleditsch in 1753.

The genus *Stemonitis* was reported to consist of 20 different species throughout the world (Lado 2005–2019), of which three species (*S. flavogenita* E. Jahn, *S. capillitionodosa* G. Moreno, D.W. Mitch., C. Rojas et S.L. Stephenson and *S. sichuanensis* B. Zhang et Yu Li) have a columella with an expanded apex (Moreno *et al.* 2010, Zhang & Li 2016). The genus *Stemonitis* is characterized by a hollow, horny or fibrous stalk and capillitium, forming a very lax internal net and a delicate complete surface net.

This paper presents morphological, ecological and geographical data on the new *Stemonitis* species and their molecular and genetic characterization. The described species are the first representatives of *Stemonitis* recorded in Russia. The new species was found on the dead wood of deciduous trees during the field surveys.

## Materials and methods

### List of abbreviations

Light microscopy (LM). Reflected light (RL). Transmitted light (TL). Scanning electron microscope (SEM). Herbarium specimens from the M.G. Popov Herbarium of the Central Siberian Botanical Garden SB RAS (NSK). Mycological Herbarium of the Komarov Botanical Institute RAS (LE).

### Morphological examination

The initial and detailed morphological examination was performed using Stemi DV4 stereomicroscope, Axiolab E-re light microscope and Zeiss Axio Imager A1 light microscope (Carl Zeiss Microscopy, Germany).

Sporocarps were preserved as permanent slides in polyvinyl lactophenol for a comprehensive examination with a LM. About 10 sporocarps and about 20 spores were measured for each species. Spores and their surface ornamentations such as spines or warts were measured and examined using the microscopy with oil immersion lens.

Sporocarps, spore ornamentations, capillitium and other microscopic structures were examined with an SEM. The SEM micrographs were produced using Carl Zeiss EVO MA 10 microscope. Specimens were air-dried and mounted on aluminium stubs with double-sided sticky film, and then sputter-coated with gold.

The nomenclature of myxomycetes used in this work follows Lado (2005–2019).

### DNA extraction and sequencing

Genomic DNA was extracted from three sporocarps of each species. The sporocarps were crushed using tissue grinding pestles in 1.5 mL centrifuge tubes using aluminium oxide (Al<sub>2</sub>O<sub>3</sub>) and then homogenized in the lysis buffer. For DNA extraction, a Phyto-Sorb kit (Synthol, Moscow) was used. The 18S rDNA (SSU) region was amplified by PCR using HS Taq DNA Polymerase (Evrogen, Moscow) and the standard pair of primers commonly used for dark-spored myxomycetes: S1F (AACCTGGTTGATCCTGCC) and SU19R (GACTTGTCCTCTAATTGTTACTCG) (Fiore-Donno *et al.* 2008). The PCR reactions were performed in C1000 Thermal Cycler (Bio-Rad, USA). Amplification results were examined using Gel Doc XR+ Imager (Bio-Rad, USA). The purified PCR products were sequenced using Big Dye terminator cycle sequence kit (ABI) and ABI Prism 3130 sequencer (Perkin-Elmer, USA).

### Phylogenetic analysis

We generated the sequences for the SSU regions of rDNA for the new *Stemonitis* species and *S. capillitionodosa*. Based on BLAST results, additional 8 SSU sequences of other *Stemonitis* species were retrieved from GenBank, which were previously published by Nandipati *et al.* (2012) and Shchepin *et al.* (2019). *Echinostelium minutum* was selected as an outgroup. The final dataset consisted of 11 SSU sequences. An overview of all taxa and on the sequences used for tree reconstruction are given in Table 1.

**TABLE 1.** Sequences of the genus *Stemonitis* used in alignment.

Species	Herbarium voucher/isolate	Genbank accession numbers
<i>S. axifera</i>	LE285125	MH930781
<i>S. axifera</i>	LE289704	MH930782
<i>S. axifera</i>	LE289730	MH930783
<i>S. axifera</i>	LE306599	MH930784
<i>S. axifera</i>	LE307469	MH930785
<b><i>S. capillitionodosa</i></b>	<b>LE306833</b>	<b>MN610391</b>
<i>S. flavogenita</i>	ATCC24714	HE614592
<i>S. flavogenita</i>	-	AF239229
<i>S. laxifila</i>	LE306548	MH930789
<b><i>S. pseudoflavogenita</i></b>	<b>LE319201 (isotype)</b>	<b>MN610392</b>
<i>Echinostelium minutum</i>	ATCC22345	HE614593

Sequences were aligned using ClustalW (Thompson *et al.* 1994). The SSU sequences were also aligned via MEGA X (Kumar *et al.* 2018). The evolutionary history was inferred by using the Maximum Likelihood method and Tamura-Nei model (Tamura & Nei 1993). The tree with the highest log likelihood (-3125.93) is shown (Fig. 3). The percentage of trees in which the associated taxa clustered together is shown next to the branches. Values on the branches represent a percentage of the 1000 bootstrap replicates, and the bootstrap values over 75% are shown on the tree. The initial tree for the heuristic search was obtained automatically by applying Neighbor-Join and BioNJ algorithms to a matrix of pairwise distances estimated using the Maximum Composite Likelihood (MCL) approach and then selecting the topology with superior log likelihood value. The tree was drawn to scale, with branch lengths measured in the number of substitutions per site. Eleven nucleotide sequences were involved in the analysis. Codon positions included 1st+2nd+3rd+Noncoding. There were a total of 615 positions in the final dataset. Evolutionary analysis was conducted using MEGA X (Kumar *et al.* 2018).

## Results

### Taxonomy

*Stemonitis pseudoflavogenita* A. Vlasenko et Novozh., *sp. nov.* Figs. 1 (A–I), 2 (E–G).  
Mycobank: MB 833184.

Description:—Sporocarps in groups, not in tufts, brown, cylindrical, with a total height of 3–5 mm and 0.25–0.3 mm wide. Hypothallus continuous under the group (confluent), very conspicuous, membranous, shiny, pale and transparent. Sporotheca is long-cylindrical. Stalk short, 0.5–1.5 mm long, 1/4–1/6 of the total height, hollow, shiny, black in reflected light and dark brown in transmitted light. Peridium fugacious. Columella always ending in a funnel-shaped expansion at the apex of the sporotheca, 50–100 µm wide, 30–50 µm height. Columella black in reflected light, dark brown and reddish-brown in transmitted light. The capillitial internal net consists of rather thick threads with many large dark brown expansions at intersections. Capillitial surface net with large meshes, consisting of rather thin light brown threads, with light expansion at intersections. Meshes mostly 25–130 µm in diameter, more often 80 µm in diameter. Capillitial threads with free ends. Capillitium is attached to the funnel-like expansion of the columella apex. Spores are free, brown in mass, light brown and orange-brown in transmitted light, globose, rough warty, 7–8 µm in diameter. Ornamentation of the surface of spores with large warts and small warts, irregularly scattered between the large warts and small indentations on the surface of the spores, that is observable only by SEM and which cannot be seen by LM. Plasmodium was not observed.

Etymology:—Referring to the resemblance to *Stemonitis flavogenita*.

Type:—RUSSIA. Krasnodar Territory, 0.5 km northwest of the village Kanevskaya, forest belt with *Fraxinus* sp., *Acer negundo* L., *Salix* sp., *Populus nigra* L., *Robinia pseudoacacia* L., on dead wood of deciduous trees, 46.107222° N, 38.931667° E, 13 m, samples collected 05 June 2017, V.N. Botyakov (holotype NSK 1026501, isotype LE 319201).

Additional specimens examined (paratypes):—RUSSIA. Novosibirsk Region, Iskitimskiy district, near the village Novososedovo, birch-aspen forest, on dead wood of *Populus tremula* L., 54.647156° N, 83.961469° E, 259 m, samples collected 01 August 2019, A.V. Vlasenko (NSK 1026481); Novosibirsk Region, near Akademgorodok, plantation with *Tilia*, on dead wood of *Tilia cordata* Mill., 54.830369° N, 84.169594° E, 151 m, samples collected 08 July 2019, A.V. Vlasenko (NSK 1026499).

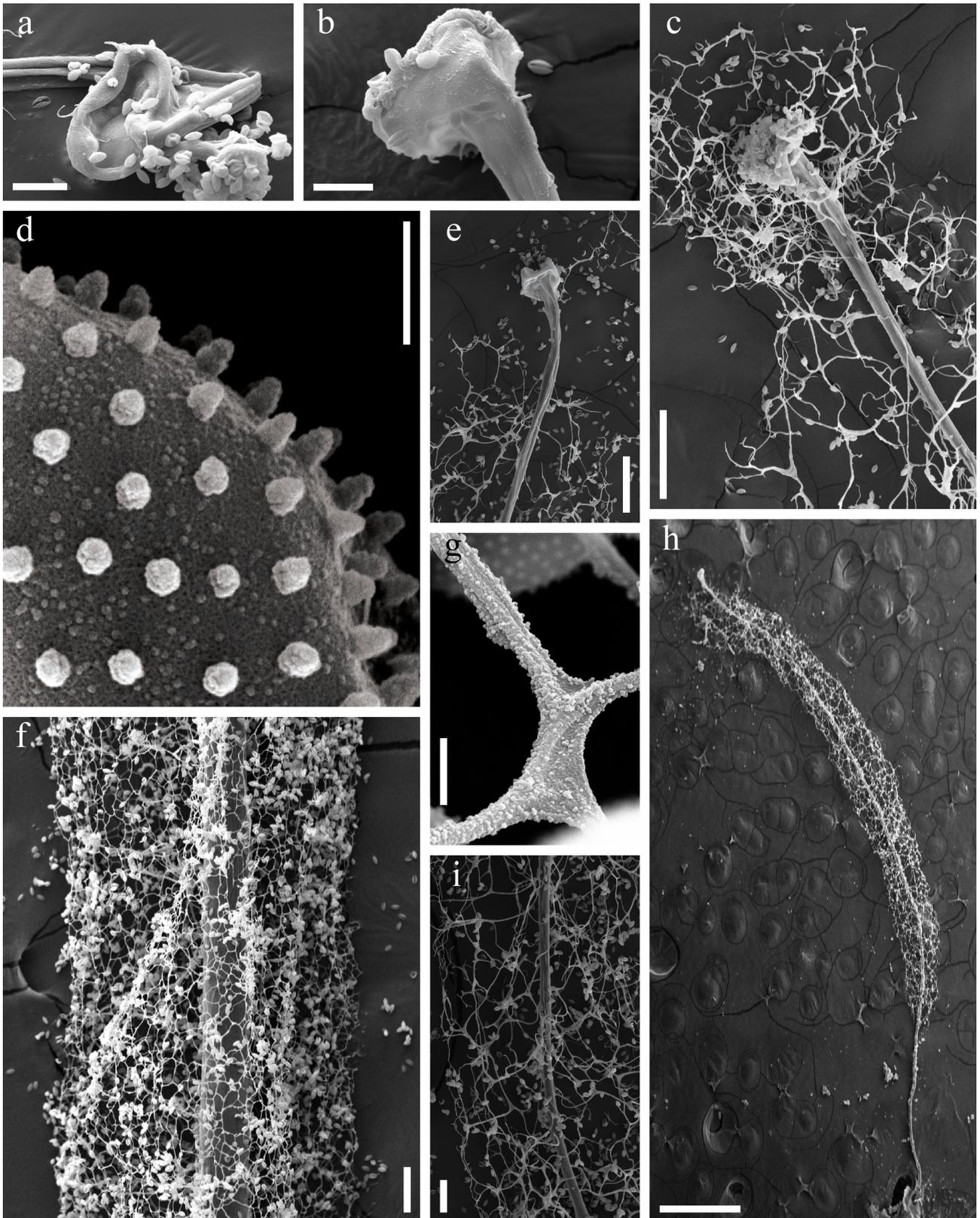
Habitat:—Dead wood of deciduous trees.

Ecology:—On dead wood.

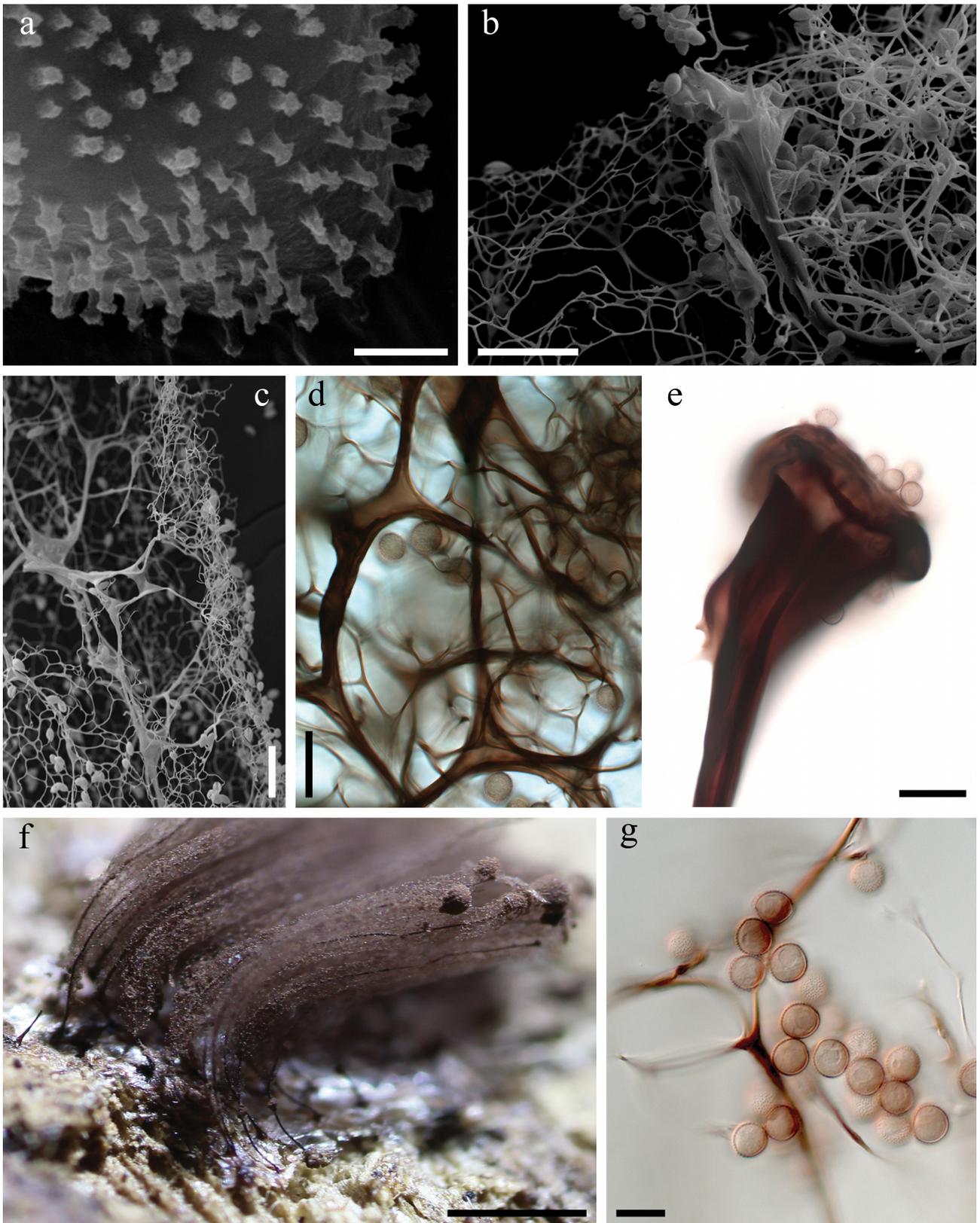
Distribution:—Currently only known from Krasnodar Territory and Novosibirsk Region, Russia.

Comments:—The species is dissimilar to any known species of the genus and clearly differs in several morphological characters (Table 2). This is a new species of *Stemonitis* characterized due to its columella apex expansion and complex ornamentation of the surface of spores consisting of large warts and small warts, irregularly scattered between large warts and small indentations on the surface of the spores. These features are observable only by SEM and cannot be seen by LM.

The brown sporocarps with columella apex expansions closely resemble those of *S. flavogenita*. However, the spores of *S. pseudoflavogenita* has more complex ornamentations, its color of hypothallus is pale, transparent (dark red-brown, rare pale in *S. flavogenita*), smaller size of sporocarps 3–5 mm in height compared to *S. flavogenita*



**FIGURE 1.** *Stemonitis pseudoflavogenita*. A, B. Apex of the columella (SEM). C. Apex of the columella and capillitial threads (SEM). D. Spore surface ornamentation (SEM). E. Apex of the columella and capillitial threads (SEM). F. Capillitium in the middle part of sporocarp (SEM). G. Capillitial threads ornamentation (SEM). H. Sporocarp (SEM). I. Capillitium at the top of sporocarp (SEM). Scale bars: A = 20  $\mu\text{m}$ , B = 20  $\mu\text{m}$ , C = 40  $\mu\text{m}$ , D = 600 nm, E = 40  $\mu\text{m}$ , F = 40  $\mu\text{m}$ , G = 1  $\mu\text{m}$ , H = 400  $\mu\text{m}$ , I = 40  $\mu\text{m}$ . Photos by: A. Vlasenko.



**FIGURE 2.** *Stemonitis capillitionodosa* (A, B, C, D), and *Stemonitis pseudoflavogenita* (E, F, G). A. Spore surface ornamentation (SEM). B. Apex of the columella and capillitial threads (SEM). C. Extensions of the capillitium (SEM). D. Extensions of the capillitium (TL). E. Apex of the columella (TL). F. Sporocarps (RL). G. Capillitial threads and spores (TL). Scale bars: A = 1  $\mu$ m, B = 40  $\mu$ m, C = 40  $\mu$ m, D = 20  $\mu$ m, E = 20  $\mu$ m, F = 1 mm, G = 10  $\mu$ m. Photos by: A. Vlasenko.

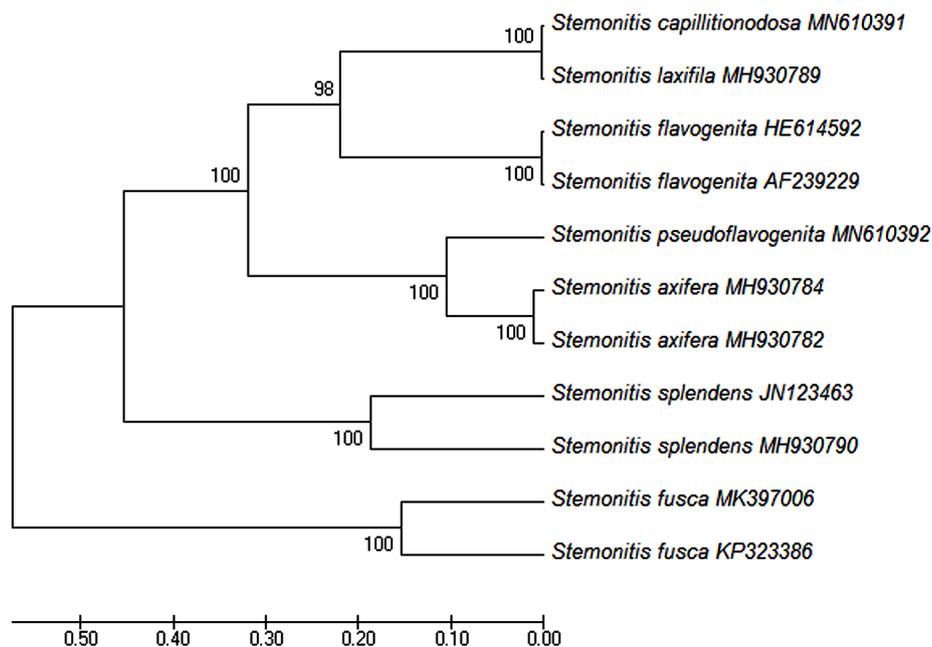
(4–10 (15) mm). The columella of *S. flavogenita* ends with a large filmy expansion, while that of *S. pseudoflavogenita* has non-filmy apex expansion.

Two other species of *Stemonitis* are known with columella apex expansions. But *Stemonitis capillitionodosa* G. Moreno, D. W. Mitch., C. Rojas et S. L. Stephenson has larger spores (9–11 µm in diameter) which are spinulose with abundant closely placed coralloid spines on surface, and reddish-brown hypothallus. *Stemonitis sichuanensis* B. Zhang et Yu Li has spores 6–7 µm in diameter, the spore surface is covered with evenly scattered small warts, hypothallus is brown, and capillitium is lacking of free ends (*S. pseudoflavogenita* has free ends at the capillitium).

Furthermore, SEM revealed that spore surface is with a variety of ornaments, which recalls *Stemonitis axifera* (Bull.) T. Macbr. But the spores of *S. axifera* have ornamentations of irregularly scattered verrucae to short bacula, and the spore surface is covered with a complete reticulum visible only with SEM (Moreno *et al.* 2013). *Stemonitis axifera* has spores 5.0–7.5 µm in diameter and brown hypothallus. We studied samples of the genus *Stemonitis* in the herbaria LE and NSK, and found that color and structure of the hypothallus are stable within species. *Stemonitis pseudoflavogenita* has confluent, very conspicuous, membranous, shiny, pale and transparent hypothallus. We believe that the samples from different herbaria previously defined as *S. flavogenita* with a shiny pale hypothallus should be redefined as *S. pseudoflavogenita*.

*Stemonitis capillitionodosa* G. Moreno, D.W. Mitch., C. Rojas & S.L. Stephenson. Fig. 2 (A–D).

Description:—Sporocarps in groups, cylindrical, total height 3–6 mm, reddish-brown. Sporotheca long-cylindrical, 2.5–3.5 mm in length and 0.1–0.25 µm in diameter. Stalk short, 1–1.5 mm long, shining, reddish-brown. Peridium fugaceous. Columella 10–20 µm wide, cylindrical, black, shining, similar in color to the stalk, often sinuous at the apex, ending in a funnel-shaped expansion 17–50 µm in diameter. Capillitium 1–2 µm in diameter, reddish-brown, shining, with a surface net consisting of variable-sized meshes (25–130 µm), with sparse spiny free ends that are more abundant at the apex of the columella, capillitial extensions abundant, angular and of variable dimensions, occasionally attached to the columella. Main branches of the very fine and sparse capillitium (1–2 µm in diameter) support the surface net and are attached to the columella. Spores 9–11 µm in diameter, globose, spinulose with abundant closely placed spines, medium violet in LM. In SEM, the ornamentation consists of dense, abundant and evenly scattered, well-developed, coralloid spines. Hypothallus continuous under the group (confluent), reddish-brown.



**FIGURE 3.** Maximum Likelihood tree based on SSU rDNA sequences showing the phylogenetic relationships between the new species of the *Stemonitis pseudoflavogenita* and other related species of the genus *Stemonitis*. Values on the branches represent a percentage of the 1000 bootstrap replicates and bootstrap values over 75% are shown in the tree. Genetic distance 0.12 with bootstrap to support *Stemonitis pseudoflavogenita* branch 100%.

**TABLE 2.** Morphological comparison among *Stemonitis pseudoflavogenita* and related species.

	Sporocarps (height, mm)	Stalk (part of the total height)	Hypothallus (color, shape)	Capillitium (free ends)	Spore (size, $\mu\text{m}$ )	Spore (ornamentation under SEM)	Columella (apex expansions)
<i>S. capillitionodosa</i>	3–6	1/3–1/4	confluent, reddish-brown	spine-like free ends	9–11	spinulose with abundant closely placed spines	present
<i>S. farrensis</i>	up to 3	1/2–1/3	confluent, brown, shining, silvery	without free ends	9–11	small warts, irregularly distributed, arranged in lines	absent
<i>S. flavogenita</i>	4–10 (15)	1/3	confluent, membranous, pale to dark red-brown	spine-like free ends	7–9	small warts, regularly distributed	present
<i>S. herbatica</i>	3–7	1/5	confluent, membranous, inconspicuous	without free ends	7–9	small angular warts, regularly distributed	absent
<i>S. mediterraneensis</i>	2.2–3.5	1/2–1/3	discoïd under the separate sporangia, yellowish-brown	without free ends	8.5–10.6	warts up to 0.3 $\mu\text{m}$ , regularly distributed	absent
<i>S. mussooriensis</i>	1.5–3	1/3–1/4	confluent, shining, silvery	spine-like free ends	10.5–12.5	warts up to 0.8 $\mu\text{m}$ , regularly distributed	absent
<i>S. pallida</i>	2–7.5	1/2–1/3	confluent, brown, or iridescent	spine-like free ends	6–8	warts up to 0.2–0.5 $\mu\text{m}$ , evenly scattered	absent
<i>S. planusis</i>	10–13	1/5	confluent, shining, membranous	without free ends	9–10	small warts, evenly scattered	absent
<i>S. pseudoflavogenita</i>	3–5	1/4–1/6	confluent, very conspicuous, membranous, shiny, pale, transparent	free ends are present	7–8	large warts and small warts, irregularly scattered between the large warts and small indentations on the surface of the spores	present
<i>S. sichuanensis</i>	10–13	1/6–1/8	confluent, brown	without free ends	6–7	evenly scattered small warts, which appears to have a slightly reticulate ornamentation	present
<i>S. splendens</i>	5–25	1/5–1/8	confluent, shining, silvery, sometimes purple	without free ends	7–9	small warts, evenly scattered	absent
<i>S. uvifera</i>	7–9	1/3	confluent, silvery	short free ends	8–10	spores firmly united in clusters of 4–12 or more, often irregular in shape, the exposed surface distinctly warted, the remainder smooth	absent

Specimen examined:—RUSSIA. Murmansk Region, Laplandskiy Biosphere Reserve, Chunozero lake, Kupletsky creek, near a waterfall, coniferous forest with *Picea obovata* Ledeb. and *Pinus sylvestris* L., 67.41017° N, 32.21525°, 191 m, samples collected 26 August 2015, Yu.K. Novozhilov, O.N. Shchepin, D.A. Erastova, E.N. Tikhonova (LE306548, LE306834).

Habitat:—Dead wood of coniferous trees.

Ecology:—On dead wood.

Distribution:—Previously known only from Costa Rica.

Comments:—We have not found any morphological differences between our specimens of *Stemonitis capillitionodosa* and the specimens described previously.

We compared the SSU rDNA sequences of *S. axifera*, *S. capillitionodosa* and *S. pseudoflavogenita*. The molecular phylogenetic analysis (Fig. 3) showed that the new species is close to *S. axifera* (voucher LE289730, GenBank No. MH930782) (Fig. 3). We made a separate comparison between the SSU sequences of *S. pseudoflavogenita* and *S. axifera*. Among a total of 400 positions in the final dataset, sequence divergences reach 21.25%, including 72 nucleotide substitutions and 6 deletions (the absence of a total of 13 base pairs). This comparison supports the establishment of the new species.

The sequence of *Stemonitis laxifila* (voucher LE306548, GenBank No. MH930789, Shchepin *et al.* 2019) was incorrectly defined as *S. capillitionodosa*. We obtained 100% similarity with the studied sample of *S. capillitionodosa* (voucher LE306833, GenBank No. MN610391). The SEM study of these samples also showed that both samples belong to the same species—*S. capillitionodosa*, which has unique ornamentation of its spores in the form of coral spines (Fig. 2 A).

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