

THE GENUS *SCOULERIA* (BRYOPHYTA) IN RUSSIA REVISITED

РЕВИЗИЯ РОДА *SCOULERIA* (BRYOPHYTA) В РОССИИ

ELENA A. IGNATOVA¹, LYUBOV E. KURBATOVA², OXANA I. KUZNETSOVA³, OLEG V. IVANOV⁴,
JAMES R. SHEVOCK⁵, BENJAMIN CARTER⁶ & MICHAEL S. IGNATOV³

ЕЛЕНА А. ИГНАТОВА¹, ЛЮБОВЬ Е. КУРБАТОВА², ОКСАНА И. КУЗНЕЦОВА³, ОЛЕГ В. ИВАНОВ⁴,
ДЖЕЙМС Р. ШЕВОК⁵, БЕНДЖАМЕН КАРТЕР⁶, МИХАИЛ С. ИГНАТОВ³

Abstract

Circumscription of *Scouleria* species in Russia is revised basing on the molecular phylogenetic analysis and morphological study. *Scouleria rschewinii* is found to have mainly western distribution in the lower course of Yenisey River in Krasnoyarsk Territory, Vilyui River and upper course of Lena River in Yakutia, and Vitim River in Irkutsk Province, but with few disjunct localities in Kolyma and Omoloy Rivers in NE Yakutia and Anadyr River in Chukotka. *Scouleria pulcherrima* is more frequent and occurs from Taimyr to Chukotka and from Baikal Lake area to the Amur River region. The traditionally used characters for differentiation of taxa are overlapping, while DNA sequence data (nuclear ITS and chloroplastic *rpl32-trnL*) and additional morphological characters (cell wall shining in polarized light, degree of peristome reduction, pattern of columella adjoining to operculum) appear to be fairly stable.

Резюме

Пересмотрены границы видов рода *Scouleria* в России на основе данных молекулярно-филогенетического анализа и изучения морфологических признаков. Показано, что *Scouleria rschewinii* имеет преимущественно западное распространение от низовий Енисея в Красноярском крае, Вилюя и верховий Лены в Якутии и Витима в Иркутской области, но с единичными находками на северо-востоке Якутии (рр. Колыма и Омолой) и на Чукотке (бассейн р. Анадырь). *Scouleria pulcherrima* встречается более часто от Таймыра до Чукотки и от Байкала до Амурской области и Хабаровского края. Традиционно использовавшиеся признаки для разграничения видов оказались широко перекрывающимися, но различия в последовательностях ДНК (ядерного маркера ITS и хлоропластного *rpl32-trnL*) и некоторых дополнительных морфологических признаках (свечении клеточных стенок в поляризованном свете, степени редукции перистома, характере прикрепления колонки к крышечке) оказались весьма стабильными.

KEYWORDS: *Scouleria*, aquatic mosses, Russia, taxonomy, phylogeny, phytogeography, ITS, *rpl32-trnL*

INTRODUCTION

Scouleria Hook. is a genus of rheophyte mosses, growing along river, creek and stream rocky banks in North Asia (Russia) eastward from Yenisey River, western North America and southern South America (Churchill, 2007; Carter *et al.*, 2014; Shevock & Norris, 2014). The genus is easily recognized by relatively large plants often forming extensive carpets on temporarily submerged rocks, and dark green color easily turning to black, especially when

dry. It often grows with *Schistidium* species of the *S. rivulare*-group, and by having superficial similarity in plant habit, and also a columella attached to operculum, *Scouleria* was originally classified in Grimmiaceae (Schimper, 1860, 1876; Brotherus, 1924). However, Churchill (1981) in his worldwide revision of the genus segregated it into its own family, Scouleriaceae S.P. Churchill. Later on, molecular phylogenetic data revealed a more distant position of this family from Grimmiaceae, placing it in a

¹ – Moscow State University, Biological Faculty, Geobotany Dept., Moscow 119234 Russia – Россия, 119991, Москва, Ленинские Горы, стр.1, корп. 12, Московский государственный университет, биологический факультет, кафедра геоботаники; e-mail: arctoa@list.ru

² – V.L. Komarov Botanical Institute Rus. Acad. Sci., Prof. Popov Str., 2, St. Petersburg, 197376 Russia – Россия 197376, г. Санкт-Петербург, ул. проф. Попова, д. 2, Ботанический институт им. В.Л. Комарова РАН; e-mail: korablik-l@mail.ru

³ – Main Botanical Garden, Russian Academy of Sciences, Botanicheskaya 4, Moscow 127276 Russia – Россия 127276 Москва, Ботаническая 4, ГБС РАН, e-mails: oikuznets@gmail.com & misha_ignatov@list.ru

⁴ – P.N. Lebedev' Institute of Physics of Russ. Acad. Sci., Leninsky 53, Moscow 119991 Russia – Россия 119991, Москва, Ленинский проспект, 53, ФИАН, e-mail: ivanov@td.lpi.ru

⁵ – Department of Botany, California Academy of Sciences (CAS), 55 Music Concourse Drive, Golden Gate Park San Francisco, CA 94118 U.S.A. E-mail: jshevock@calacademy.org

⁶ – Department of Biology, Duke University, Durham, North Carolina 27708, U.S.A.; e-mail: benjaminrcarter125@gmail.com

separate order, Scouleriales, with two families, Scouleriaceae and Drummondiaceae (Goffinet, 2009).

The taxonomy of *Scouleria* at the species level has been challenged several times in last decades, and is still not completely understood, as these aquatic plants exhibit enormous variation. Six species were described within the genus: *S. aquatica* Hook., *S. marginata* E. Britton and *S. siskiyouensis* Shevock & D.H. Norris from North America, *S. patagonica* (Mitt.) A. Jaeger from South America, and *S. rschewinii* Lindb. & Arnell and *S. pulcherrima* Broth. from North Asia.

Scouleria rschewinii was described based on collections made by H.W. Arnell the lower course of Yenisey River, Tolstoi Nos and Igarskoe, during the Swedish expedition to Yenisey River in 1876. The name was given in honor of the privy councillor Rschewin who was an attendant of this expedition and who had shown a locality of *Scouleria* to Hampus Arnell. Later V.F. Brotherus described another species, *S. pulcherrima*, from the collection of Olga Kuzeneva from Zeya River basin (Amurskaya Province, Russian Far East). The main difference between these species was indicated as a presence of bistratose submarginal border in *S. pulcherrima*, which was absent in *S. rschewinii*. Both species were accepted by Savicz-Lyubitskaya & Smirnova (1970) who mentioned a wider distribution of *S. rschewinii* (Siberia and Far East), while *S. pulcherrima* was known only in the Russian Far East; although both species were considered to be rare, always lacking sporophytes. The diagnostic characters used in the key included laminal cells size, 9–12 µm in *S. rschewinii* versus 12–15 in *S. pulcherrima*, and presence of submarginal border of elongate cells in the latter species.

Churchill (1985) revised the genus worldwide and accepted only three species in the world. He synonymized both Asiatic *S. rschewinii* and *S. pulcherrima* with the North American *S. aquatica*. Note that the range of the latter species reaches Alaska, thus coming in close proximity with *Scouleria* in Asia, where the genus penetrates to the north up to Chukotka. Churchill's conclusion was based on a great variability of morphological characters in Asiatic and North American specimens. This concept was generally followed by Kurbatova (1998) who summarized all the known data about the distribution of *Scouleria* in Russia and also briefly described sporophytes found in some collections. Kurbatova agreed with the synonymization *S. rschewinii* with *S. aquatica*, but *S. pulcherrima* has been recognized by her within *S. aquatica* at the level of variety, *S. aquatica* var. *pulcherrima* (Broth.) Kurbatova. According to Kurbatova (l.c.), var. *pulcherrima* differs from the type variety by the unique bistratose submarginal border composed of elongate cells and extending from leaf base to its middle part, as well as by elongate shortly exerted capsules on longer seta (vs. immersed spherical capsules of type variety). Distribution of var. *pulcherrima* has been described as much

more restricted in comparison with var. *aquatica*: it included Amurskaya Province, two localities in Yakutia and several localities in Chukotka, while var. *aquatica* was currently known from Krasnoyarsk Territory (type locality of *S. rschewinii*), many places throughout Yakutia, from Buryatia and Chita Province, Amurskaya Province, Khabarovsk Territory, and Chukotka.

The next attempt to clarify the species limits and their relationships within *Scouleria* has been made by Carter *et al.* (2014) who provided a molecular study of the genus based on nuclear ITS and three chloroplast loci. They confirmed species status of *S. patagonica* and *S. marginata*, pointed on one undescribed species with a narrow distribution in Oregon, U.S.A. (described shortly afterwards by Shevock & Norris, 2014 as *S. siskiyouensis*). In addition, their molecular data suggested a resurrection of *S. rschewinii* and *S. pulcherrima*, which were found to be distinct from North American specimens of *S. aquatica* in their molecular phylogenetic analysis. In total, 7 specimens from north Asia were sequenced, 6 of them were referred to *S. pulcherrima* and one to *S. rschewinii*. These data indicated a different morphological circumscription of *S. pulcherrima*, because specimens with submarginal border composed of elongated cells were grouped with specimens lacking such border.

All the above mentioned discrepancies forced us to conduct an additional morphological and molecular study of *Scouleria* specimens from Russia in order to clarify their taxonomy and distribution pattern in Asia.

MATERIAL AND METHODS

Molecular phylogenetic study. 15 specimens of *Scouleria* from Russia were added to the set used by Carter *et al.* (2014) for their molecular study, thus the total number of the specimens from Asiatic Russia reached 22, while the whole set was of 41. Label data and Genbank accession numbers for the additionally sequenced specimens are given in Appendix 1.

Nuclear ITS and chloroplastic *rpl32-trnL* were taken, as they provide the stronger phylogenetic signal.

Laboratory protocol was essentially the same as in previous moss studies, described in detail by, e.g., Gardiner *et al.* (2005). Sequences were aligned by Clustal and modified manually using BioEdit 7.0 (Hall, 1999).

Preliminary phylogenetic analyses of the chloroplast and ITS datasets indicated incongruence only at a single basal node (see Carter *et al.*, 2014 for discussion) that was not relevant to the present study and so the chloroplast and ITS datasets were combined. Bayesian analysis was conducted in MrBayes (Huelsenbeck & Ronquist, 2001) using the GTR+G model as selected by MrModeltest2.3 (Nylander, 2004) and employing the combined chloroplast and ITS dataset. The Bayesian analysis ran for 10⁷ generations with sampling every 1000 generations. Two simultaneous runs were used, each with three hot chains and one cold chain. Stationarity was assessed by confirming that the average standard deviation of split frequencies

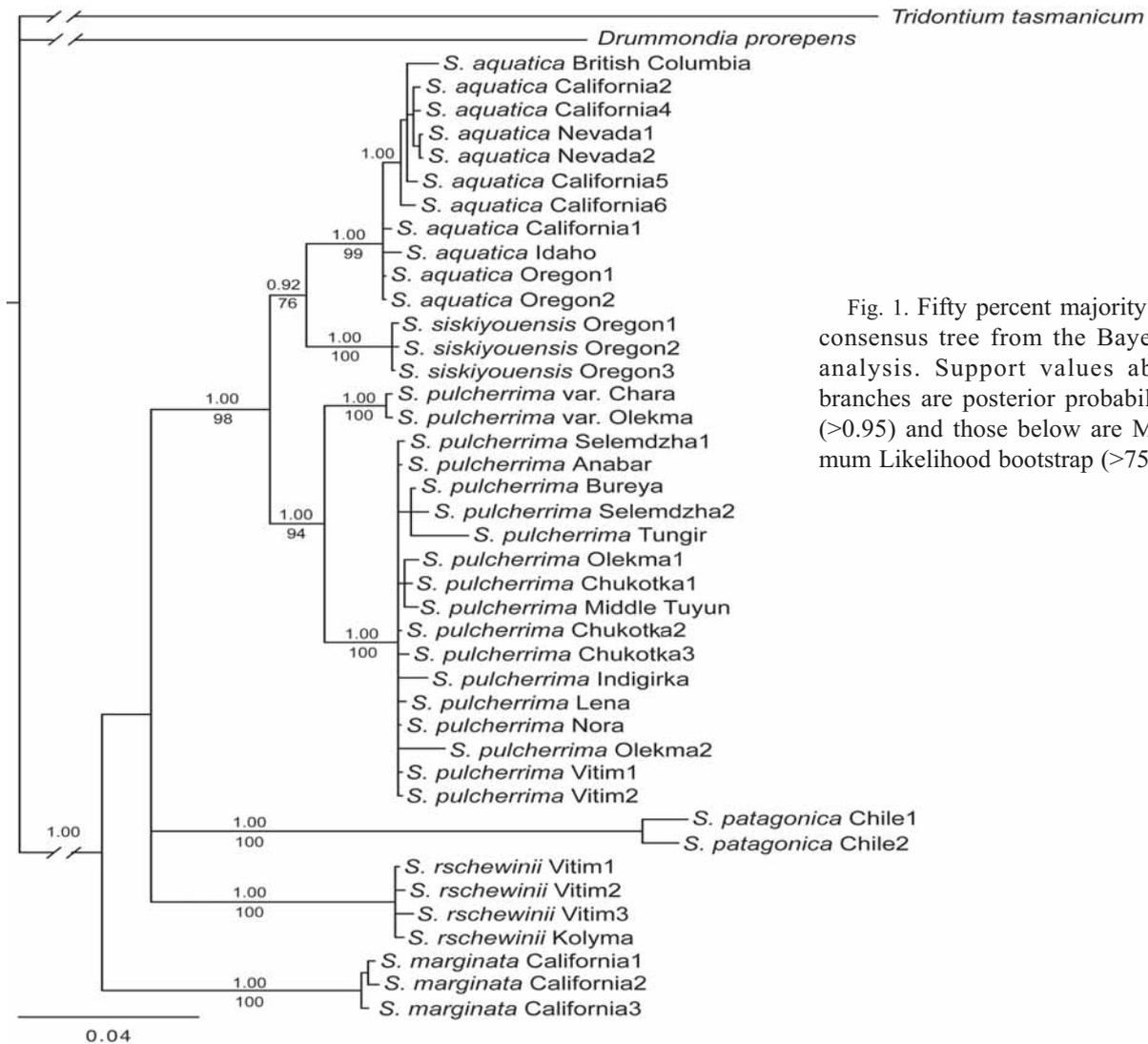


Fig. 1. Fifty percent majority rule consensus tree from the Bayesian analysis. Support values above branches are posterior probabilities (>0.95) and those below are Maximum Likelihood bootstrap (>75).

was below 0.01 and by graphing the log likelihood scores for each run. The first 25% of sampled trees were discarded for the burn-in. A separate Maximum Likelihood analysis was conducted on the combined dataset, also with the GTR+G model, implemented in RAxML 8.0 (Stamatakis, 2014) with 1000 bootstrap pseudoreplicates.

Morphological study. We revised all *Scouleria* specimens from MHA, MW, LE and SASY (altogether from ca. 60 localities). Isolectotype of *S. rschewinii* (H-Br 3810004, LE) and lectotype of *S. pulcherrima* (H-Br 3810002) were also studied.

Three characters previously used for differentiation of *S. rschewinii* and *S. pulcherrima* were estimated for each specimen: 1) presence of bistratose marginal or submarginal patches or strips; 2) presence of submarginal border composed of 3–5 rows of elongate cells in lower part of leaf and its extending upward; 3) laminal cell size. Presence of marginal border formed of several rows of darker cells, being more thick-walled and thicker albeit unistratose (e.g., Figs. 4, 9–11), was also specially addressed.

In order to check if *S. rschewinii* and *S. pulcherrima*

differ in cell size, three specimens of the former species and seven specimens of the latter were studied with the use of the program AREOANA (Ivanov & Ignatov, 2011, 2013). Leaves were photographed under Carl Zeiss NU2 light microscope, using the Nikon D70 camera (2000×3008 pixel). Three frames with polarized filters at 0°, 30° and 60° angles were taken for each image, and their combined image provided a polarized light “staining” of all cell walls, following the algorithm developed before (Ivanov & Ignatov, 2011, 2013). In total, 15 leaves from 11 specimens were studied (Table 1), with ca. 45000 cells studied for their dimensions. Several images of one leaf taken with a certain overlap were assembled by internal tool of AREOANA program (<http://arctoa.ru/areoana/>) after the cell outline recognition. After cell outline digitizing (algorithm is described by Ivanov & Ignatov, 2011) cell squares and dispersions were calculated.

For peristome studies capsules were soaked in water, cut into two halves and photographed under Olympus SZX16 stereomicroscope and CX41 compound microscope, under the latter partly in transmitted light, partly

using fluorescent microscopy observation with berberin staining, $\lambda = 488$ nm. Autofluorescence of lamina cells was studied also with LCSM Olympus FV-1000.

RESULTS

Molecular phylogenetic results

In general, the topology presented here agrees with analyses of an earlier dataset with fewer accessions but more chloroplast data (Carter *et al.*, 2014). The most important difference is the placement of the Asiatic species *S. rschewinii*. In this analysis, four vouchers representing *S. rschewinii* form a supported clade outside of the crown clade comprising the North American *S. aquatica* and *S. siskiyouensis* and the Asian *S. pulcherrima*. The single voucher used for *S. rschewinii* by Carter *et al.* (2014) has been re-examined morphologically for this study and is *S. pulcherrima* (*S. pulcherrima* var. *Olekma* in Fig. 1). That specimen from the Olekma River in SW Yakutia along with another from the nearby Chara River form the sister group to a larger and more widespread *S. pulcherrima* within the crown *S. aquatica* clade. Specimens morphologically assigned to *S. rschewinii* in this study were resolved as the third lineage of the polytomy that includes also *S. marginata* and *S. patagonica*. These specimens were from the Vitim and Kolyma Rivers (cf. Fig. 8), where *S. pulcherrima* also occurs. We therefore recognize two species of *Scouleria* in Asia, the somewhat narrowly distributed *S. rschewinii* and the more widespread *S. pulcherrima* which comprises two morphologically indistinguishable subclades.

Morphological study

Molecular data pointed the heterogeneity of Siberian material, so morphological study has been addressed to confirm or reject a number of hypotheses on the identity of three main entities found in our phylogenetic analysis, namely (1) the strongly differentiated Vitim+Kolyma population; (2) moderately differentiated Chara+Olekma population, and (3) the rest of plants.

The possible resolution could be as follows:

1) Vitim+Kolyma population belongs to new undescribed species; Chara+Olekma populations belong to *S. rschewinii*; the rest belong to *S. pulcherrima*.

2) Vitim+Kolyma population belongs to *S. rschewinii*; Chara +Olekma population is a marginal genetic variation within *S. pulcherrima*, where all other Siberian populations also belong.

3) Vitim+Kolyma population belongs to new undescribed species; Chara+Olekma population belongs to the second undescribed species; *S. rschewinii* is known only from type locality in Lower Yenisey River; the rest of Siberian *Scouleria* belongs to *S. pulcherrima*.

4) Minor variations in DNA also require recognition at taxonomic level, so Asiatic *Scouleria* is represented by 5 or more species.

5) DNA data are extremely incongruent with morphology, and no reliable characters can be found to support molecular differentiation of taxa, thus the only pos-

sible way is to retain all populations within one species until good characters for species identification will be found, or at best segregate plants with better developed border as a separate taxon of this or that level.

1. Marginal and submarginal border (Figs. 8-11)

Speaking of the (sub)marginal border in Asiatic species of *Scouleria*, the authors usually mean three different things. The border may be differentiated of cells longer than others from the leaf surface view, or it is bistratose (in two American species multistratose), and also cells near leaf margin may be enlarged. In the latter case submarginal cells are enlarged especially in their height, which is clearly seen in leaf transverse section, although often it can be seen just from the surface view, by slightly adjusting the fine focus of compound microscope. In addition, these enlarged cells often develop brownish pigmentation. In fact, polarizing light (cf. Figs. 3, 4) provides another definition of the border, as a shining zone near margin. All these types of border expression are congruent between each other only to a certain extent: elongate cells might be unistratose or bistratose, and the way of differentiation in mid-leaf might be slightly different than below, where the border is usually more pronounced by all of these three meanings.

The type of *S. rschewinii* lacks any border (Figs. 4: b,d; 12). The type of *S. pulcherrima* has a relatively moderately developed border: in the lower part of leaves cells are occasionally bistratose in 1–2 rows, but not always. In unistratose leaves, however, cells are larger towards the margin, with the largest being at the distance 2–5 cells from margin, which is clearly seen in leaf transverse sections (Figs. 9–10). On the surface view, the cells are strongly elongated near leaf base, while in the middle of leaf the submarginal border is composed of more or less isodiametric cells, but is still quite distinct, as these cells are darker and often with brown pigmentation in cell walls. In the studied populations of the species, the border sometimes is bistratose up to 4–5 rows and the longer cells are reaching the mid-leaf, although usually they are becoming short at about 0.2–0.3 of leaf length. Quite often longer cells are hardly discernable near leaf base. There are populations (some collections from the Anabar River), where the submarginal border is poorly developed. Specimens from the Chara-Olekma population have rather poor border development: longer cells are not apparent, although leaves have 1–2-rowed bistratose strips in lower part, or some leaves are unistratose throughout.

The approach to the border as is it seen in polarizing light is discussed below.

2. Cell size (Fig. 2, Table 1)

Measurement of some individual cells with the ocular-ruler or with digital photographs only partly supports the previous idea about the species distinction. In the type of *S. rschewinii*, cells are smaller than in the type of *S. pulcherrima*, but even in the type specimen of *S. rschewinii*, in some leaves cell are larger than 12 μm ,

| Specimen | N_cells | CELL AREA | | | CELL LENGTH | | | CELL WIDTH | | | |
|--|---------|-----------|-------|-------|-------------|-------|-------|------------|-------|-------|-------|
| | | Mean | Sigma | V_min | Mean | Sigma | V_min | Mean | Sigma | V_min | |
| <i>rschewinii</i> _Vitim_Bardunov_10.VII.1984_a | 3135 | 109.52 | 36.46 | 50.56 | 171.07 | 12.99 | 8.62 | 10.99 | 1.99 | 6.92 | 14.95 |
| <i>rschewinii</i> _Vitim_Bardunov_10.VII.1984_b | 3091 | 115.46 | 47.48 | 42.37 | 196.03 | 14.10 | 9.36 | 11.40 | 2.35 | 6.96 | 15.80 |
| <i>rschewinii</i> _Vitim_Mamontov_464/1_a | 2476 | 124.54 | 44.14 | 49.78 | 200.71 | 14.24 | 9.40 | 11.67 | 2.33 | 7 | 15.99 |
| <i>rschewinii</i> _Vitim_Mamontov_464/1_b | 2008 | 134.06 | 51.26 | 59.92 | 224.11 | 15.36 | 10.62 | 12.41 | 2.34 | 8.07 | 16.88 |
| <i>rschewinii</i> _Yenissey isotype LE | 1879 | 121.14 | 47.89 | 49.39 | 204.22 | 14.54 | 9.96 | 11.89 | 2.26 | 7.59 | 16.28 |
| <i>rschewinii</i> _Yenissey isotype H-SOL | 3923 | 124.91 | 61.46 | 40.42 | 239.71 | 14.92 | 9.59 | 12.03 | 2.84 | 7.03 | 17.43 |
| <i>rschewinii</i> _Ust-Belaya_Afonina_19.8/1980 | 2711 | 125.57 | 44.94 | 59.14 | 201.10 | 14.70 | 10.33 | 11.84 | 2.16 | 7.66 | 16.06 |
| <i>pulcherrima</i> _Anabar_Fedosov_08-344 | 3040 | 125.26 | 43.26 | 60.31 | 201.49 | 14.90 | 9.99 | 11.73 | 2.05 | 7.77 | 15.84 |
| <i>pulcherrima</i> _Tungur_Golyakov_21.7.1996 | 3774 | 130.69 | 47.79 | 62.26 | 213.97 | 14.92 | 9.88 | 11.94 | 2.22 | 7.70 | 16.28 |
| <i>pulcherrima</i> _Selemdzha_Bezgodov_171 | 3921 | 144.07 | 53.21 | 61.48 | 233.86 | 15.67 | 10.55 | 12.89 | 2.44 | 8.40 | 17.47 |
| <i>pulcherrima</i> _Indigirka_Nikolin_27.7.2009_a | 3178 | 147.73 | 54.92 | 64.60 | 240.10 | 16.33 | 10.95 | 12.60 | 2.52 | 7.85 | 17.25 |
| <i>pulcherrima</i> _Chara_Krivoshapkin_VIII.1996_a | 2365 | 152.38 | 50.69 | 76.69 | 239.32 | 16.14 | 11.10 | 13 | 2.23 | 8.73 | 17.39 |
| <i>pulcherrima</i> _Byreya_Kurbatova_4.07.1996 | 3042 | 150.94 | 57.04 | 62.65 | 253.36 | 15.94 | 10.55 | 13.04 | 2.70 | 8.03 | 18.06 |
| <i>pulcherrima</i> _Anabar_Fedosov_07-222 | 3325 | 150.96 | 59.91 | 60.31 | 257.26 | 16.55 | 11.07 | 13.41 | 2.68 | 8.55 | 18.35 |
| <i>pulcherrima</i> _Indigirka_Nikolin_27.7.2009_b | 2443 | 158.54 | 49.49 | 85.27 | 243.22 | 16.60 | 11.36 | 12.99 | 2.11 | 8.88 | 17.13 |
| <i>pulcherrima</i> _Chara_Krivoshapkin_VIII.1996_b | 2565 | 177.38 | 55.83 | 95.02 | 277.93 | 17.35 | 12.06 | 13.95 | 2.28 | 9.62 | 18.35 |

Table 1. Cell size of *Scouleria rschewinii* and *S. pulcherrima* calculated in AREOANA, compare with Fig. 2 (a/b means different leaves of same specimen).

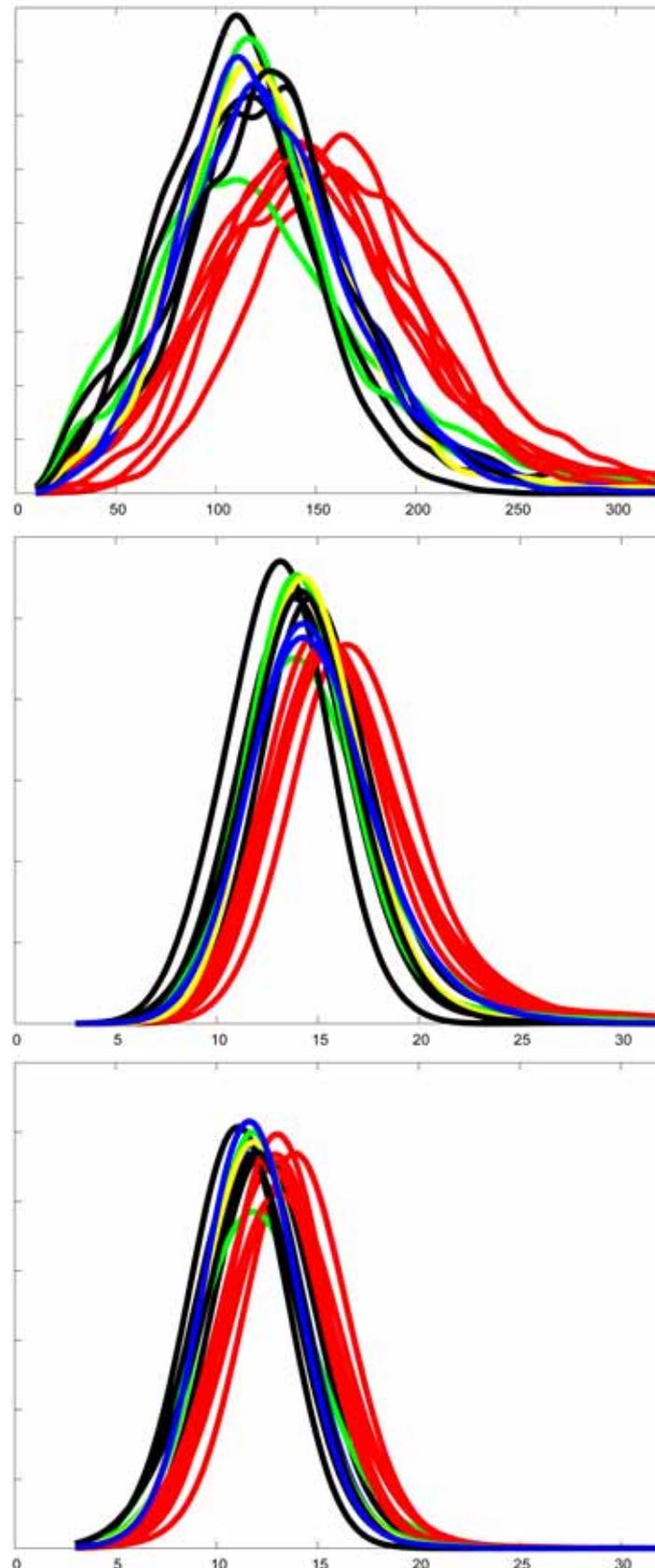


Fig. 2. Cell size of *Scouleria rschewinii* and *S. pulcherrima*: A: area, B: length; C: width. Axis X shows dimension in square microns (A) and μm for B and C. Axis Y: arbitrary units, made so that the square under distribution curve is equal to 100%. Specimen colors are specified in Table 1.

while Lindberg & Arnell (1890) and Savicz-Lyubitskaya & Smirnova (1970) report 9–12 μm for this species. Especially problematic is *S. pulcherrima*, where cells are very irregular in shape and size.

After an application of the AREOANA method with counting several thousand cells, the following distribution of cell size was obtained (Fig. 2).

Cells in two specimens from Vitim River are the smallest and the distribution is narrowest, so their peaks are the highest (black in Fig. 2). Most specimens of *S. pulcherrima* exhibit a fairly close distribution, being both the largest and most variable (red in Fig. 2). There are, however, some specimens that make this difference between extremes not so disruptive. The type of *S. rschewinii* has smaller cells with a rather broad variation, so peaks of its leaves is under those measured for the Vitim population, but broader. The most confusing are a few specimens of *S. pulcherrima* from different areas, that have smaller cells (blue in Fig. 2), which preclude differentiation of the group into contrasting entities. Such specimens have usually a well-developed border, which likely provide a more regular and smaller areolation in this species.

3. Cell appearance in polarizing and fluorescent microscopy (Figs. 3–4)

Although the dimension study with polarizing microscopy gave no straightforward delimitation between taxa of *Scouleria*, it highlighted an interesting peculiarity of *Scouleria*. Cell walls are hardly seen in most part of leaf in most populations studied by DNA (Fig. 3c), except the Vitim (Figs. 3a) and Kolyma plants. However the type of *S. rschewinii* (Figs. 3b) is similar to Vitim in this respect.

Polarizing images also highlight the specific arrangement of cells in blocks (Frey, 1970) 4×4, 4×8, etc. which is clearly seen in *S. rschewinii* and is not apparent in *S. pulcherrima* (Fig. 4). This pattern is also seen under light microscopy as thicker walls between cell blocks, indicating different way of development. Possible explanation of these differences are that the cell walls in *S. rschewinii* undergo thickening shortly after their formation, providing a basic frame where cells are somewhat compartmentalized, in contrast to *S. pulcherrima*, where cell block limits are unseen, apparently due to more gradual process of cell wall formation.

Autofluorescence studies of both the recently collected material (Fig. 3: a5, c5) and XIXth century collections (Fig. 3: b5, d5) demonstrate apparent corner thickenings in *S. rschewinii* and also different pattern of light absorption. However, a comparison of the latter aspect should be done among specimens of the same age only. In recent collections cell walls are much darker in *S. pul-*

cherrima than in *S. rschewinii* (Fig. 3: a5 and c5). In old collections cells walls are very dark in both species, but in *S. rschewinii* a light-transparent material appears to be very dark in fluorescence, making striking difference of cell outlines, which was not seen in *S. pulcherrima*. The physical reasons for that and parallels to what is seen in polarized light need a special study.

4. Capsule shape and seta length

Capsules were found in only 13 populations. We found no significant difference in seta length between two species. However, the shape of capsules, especially in wet condition, appeared to be different. When dry, capsules of *S. rschewinii* from the Vitim plants (Fig. 5: a, c) and one collection from Chukotka (Belaya River) are more flattened than those of *S. pulcherrima*, and after wetting capsules of the Vitim and Ust-Belaya plants become very low cylindrical (Fig. 5: f), contrary to globose in the latter species (Fig. 5: g).

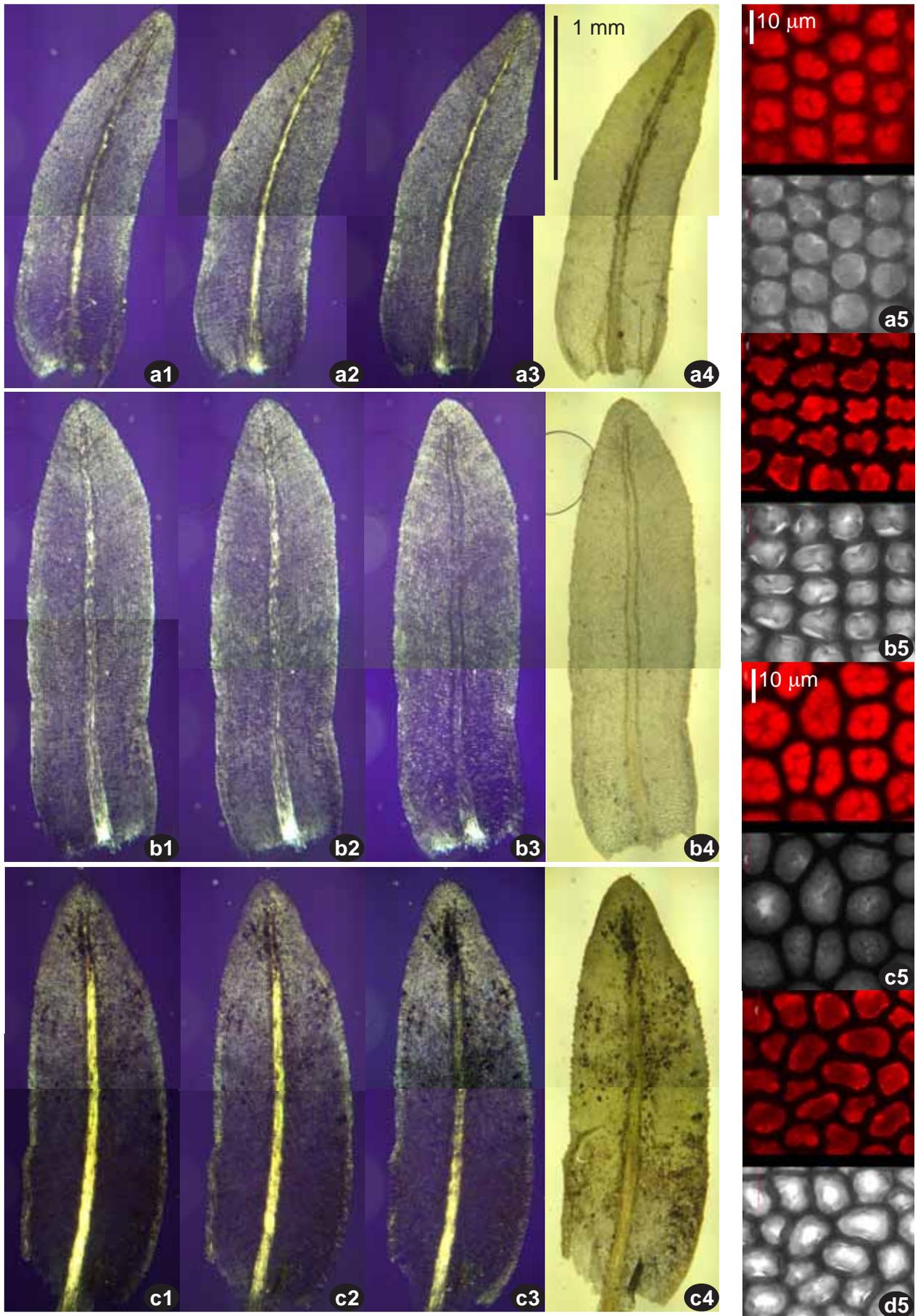
5. Columella and peristome

Capsules of *Scouleria* are referred to a systylious type, i.e., columella remains attached to the operculum after the dehiscence and spore release. However, contrary to many other systylious mosses, in *Scouleria* columella does not fall off, and after wetting it shortens, while the capsule walls are enlarging, so the capsule is achieving globose shape with operculum closing the mouth, as it was before the capsule dehiscence. Columella has a widening at the upper level of the spore sac, forming “epigramm”, which is appearing just below the peristome. As peristome formation in arthrodontous mosses is always associated with partial cell wall resorption, the “paraperistomial cavity” is forming in between the epiphragm and operculum wall.

Capsules from the Vitim and Ust-Belaya and the rest of collections show rather different structure when they are cut longitudinally in half (Fig. 6: a, c). In *S. pulcherrima*, the species with a rather normally developed teeth, the band between epiphragm and operculum is narrow (Fig. 6: a, b), while in the Vitim and Ust-Belaya plants, whose peristomes are poorly developed, epiphragm is attached to the operculum by its whole distant part (Fig. 6c,d). Superficially this difference is observable in opened capsules as the presence (in *S. pulcherrima*, Fig. 5: b, d) or absence (in the Vitim and Ust-Belaya plants, Fig. 5: e) of widening at a certain distance from the operculum.

Peristome in the Vitim and Ust-Belaya plants is reduced in a specific way. In open capsules it is almost unseen (Fig. 6: d) or represented by lowermost plates or otherwise broken teeth (Fig. 7: d, e). On the lower surface of the operculum the brownish spots are seen (Fig.

Fig. 3 (opposite page). Cell difference between *Scouleria rschewinii* and *S. pulcherrima*: a – specimen from Vitim population, Mamontov 464/1, LE; b – isotype of *S. rschewinii*, LE; c – Selemdzha River, 11.VI.2011 *Bezgodov* #171, MHA; d – isotype of *S. pulcherrima*, LE. Indexes 1–3 indicate polarized light photos, taken at 0°, 30° and 60° angle of polarizing filter (note that 90° is same as 0°); under index 4 are ordinary transmitted light photographs; 5 shows autofluorescence in combined 405, 473, 576 nm lasers, in red and pseudo-transmitted light channel of LCSM: note difference in corner thickenings and cell outlines between species due to different pattern of light absorption.



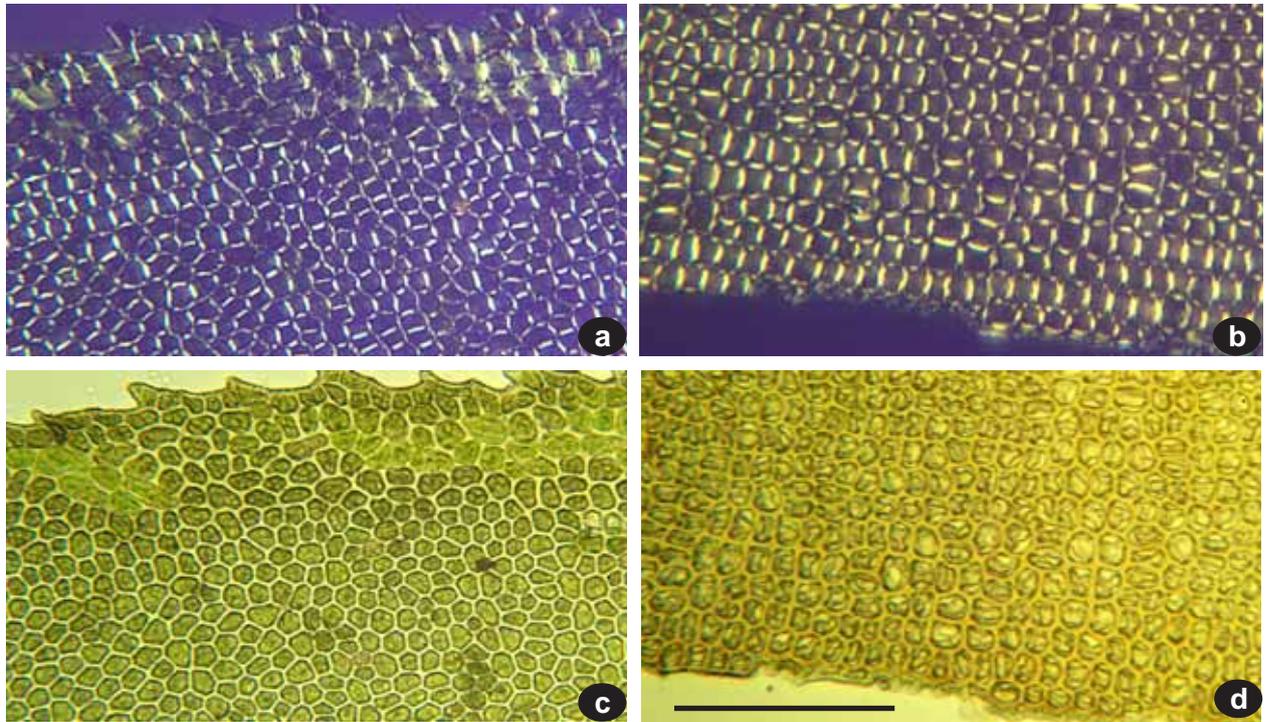


Fig. 4. Areolation pattern difference between *Scouleria pulcherrima* (a, c) and *S. rschewinii* (b, d); a, c – from: Amurskaya Province, 11.VI.2011, *Bezgodov 171*, MHA; b, d – from isotype of *S. rschewinii*, LE. Photos a and b are made in polarized light. Note (1) the difference in shining of cell walls of marginal and mid-leaf cells in a and uniform shining of all cells in b; (2) darker leaf margin in c and its absence in d; (3) more or less regular blocks of square cells in b and d, while hexagonal cells in a and c do not show subdivisions into blocks. Scale bar: 100 μ m for all.

7: a-c), looking rather as “ghosts” of teeth than anything reasonably solid for functioning. However, fluorescent microscopy discovers peristome in much better shape, allowing for conclusion that periclinal cell walls are still there, but they cannot be observed in the ordinary microscope due to their transparency (cf. Fig 7: b). Deposition of additional cell wall material occurs only at places, and the distally, the weaker.

Spores in *Scouleria* may propagate partly inside the capsule, the character similar to phylogenetically closely related *Drummondia*. Spores are very variable in size, so the difference between species can be found only by mass-measurements and statistical study, but is unlikely helpful for practical taxonomy.

DISCUSSION

Returning to the main question addressed in the introduction, the hypothesis of the total incongruence of molecular and morphological data has to be rejected: all most distinct molecularly specimens of *Scouleria* from the Vitim and Kolyma populations have laminal cell walls strongly shining in polarizing light. At the same time, the only sequenced plant with capsule with reduced peristome was one from molecularly peculiar Vitim plants. In addition, all these specimens have almost undifferentiated submarginal border (Fig. 4) and smaller laminal cells. Two latter characters have a great overlap with *S. pulcherrima*, but, nonetheless, the tendency is quite apparent (e.g. in Fig. 2).

The problem if the type of *S. rschewinii* and the Vitim

plus Kolyma populations belong to the same species cannot be solved at a hundred percent reliable basis, as there is no collection from the lower course of Yenisey River since 1876 suitable for sequencing. All original collections of this species lack sporophytes, thus especially conspicuous distinction in peristome structure cannot help in this case as well. The polarizing light is the most important character, as the specific shining is known in the Vitim population and the type of *S. rschewinii*, whereas in all sequenced populations of *S. pulcherrima* specimens were found to be dark in such investigations (Fig. 3). Also, the same cell size and lack of border support their close relationship. Thus, we consider Vitim plus Kolyma plants and the type of *S. rschewinii* conspecific, and therefore accept this name for Vitim and Kolyma plants, and for Ust-Belaya population due to its subidentical with Vitim plants peristome and columella, and also for some other populations with similar morphological characters (Fig. 8), especially shining cells in polarized light.

It turned out that *S. rschewinii* occurs mainly along the western edge of *Scouleria* distribution in Siberia: from the lower course of Yenisey River, along Vilyuj and upper course of Lena River in Yakutia, and the Vitim River in Irkutsk Province. However a few disjunct localities were found in Kolyma and Omolj Rivers in NE Yakutia and in Anadyr River basin in Chukotka. At the same time, the majority of Asiatic populations belong to *S. pulcherrima*, which is locally common from Taimyr to Chukotka and

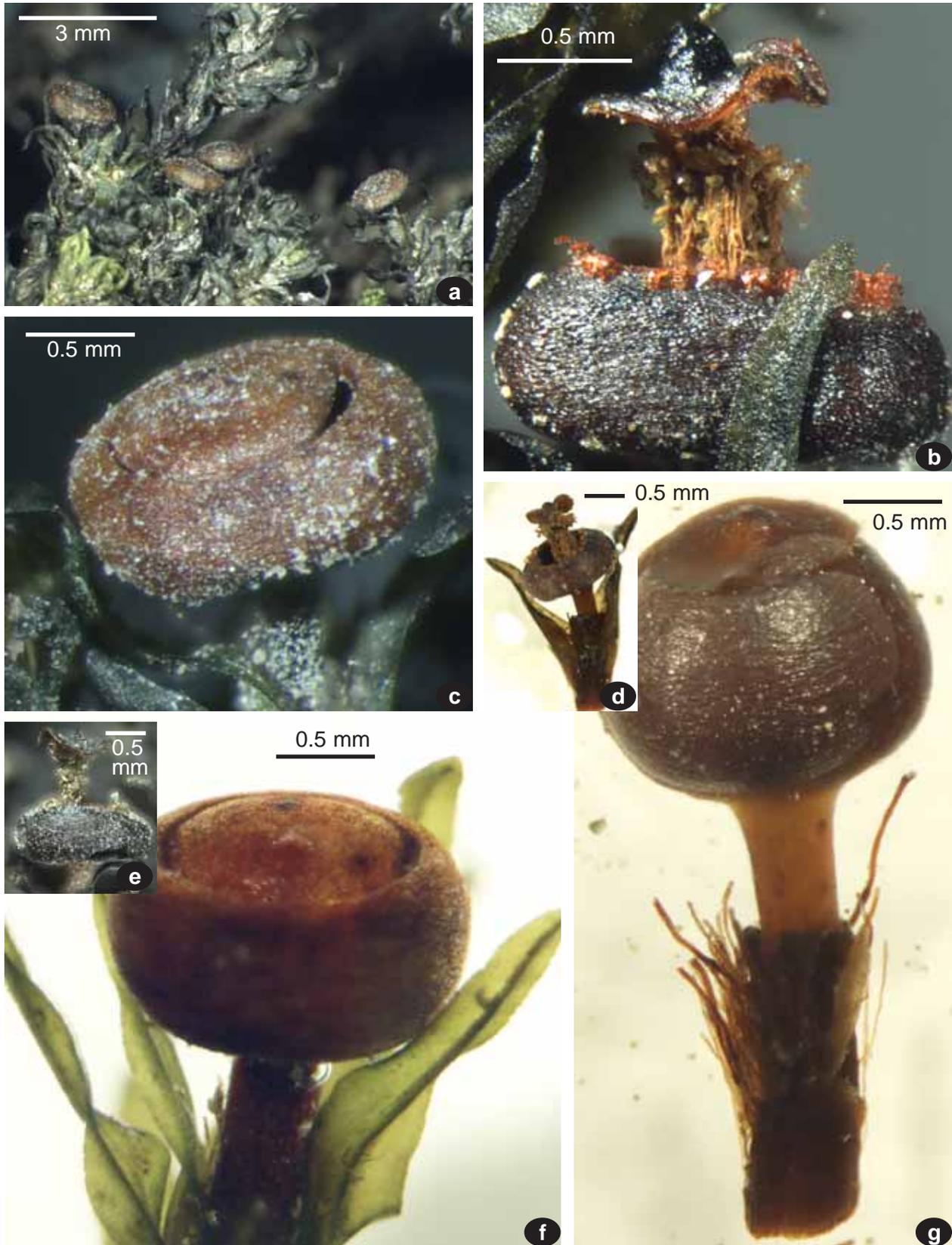
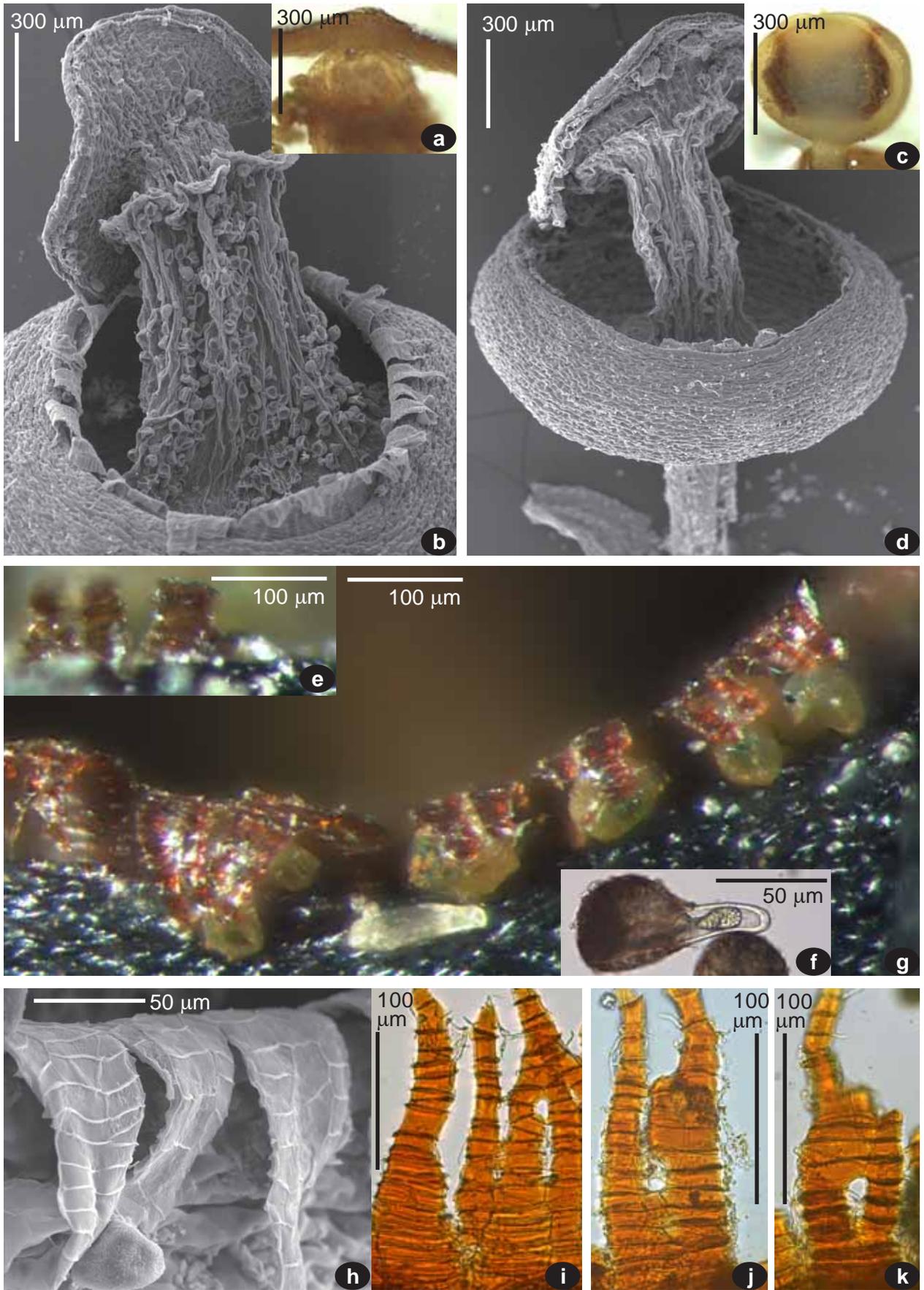


Fig. 5. Capsules of *Scouleria rschewinii* (a, c, f – from Russia, Irkutsk Province, Vitim, 10.VII.1984, *Bardunov s.n.*, MHA); e – from Irkutsk Province, Verkhny Yuryakh Creek, 18.VII.1984, *Bardunov s.n.*, LE) and *S. pulcherrima* (b – from Zabaikalsky Territory, Tungir River, Baldyk Creek mouth, 2000, *Golyakov s.n.*, LE; d, g – from Chukotka, Anadyr River basin, Golubaya River – tributary of Tanyurer River, 13.VIII.1979, *Afonina, Bryophyta Rossica exsiccata 186*, LE). Capsule “f” is the same as “c” after wetting; and “g” is the same as “b” and “d”; “d” shows a widening of columella.



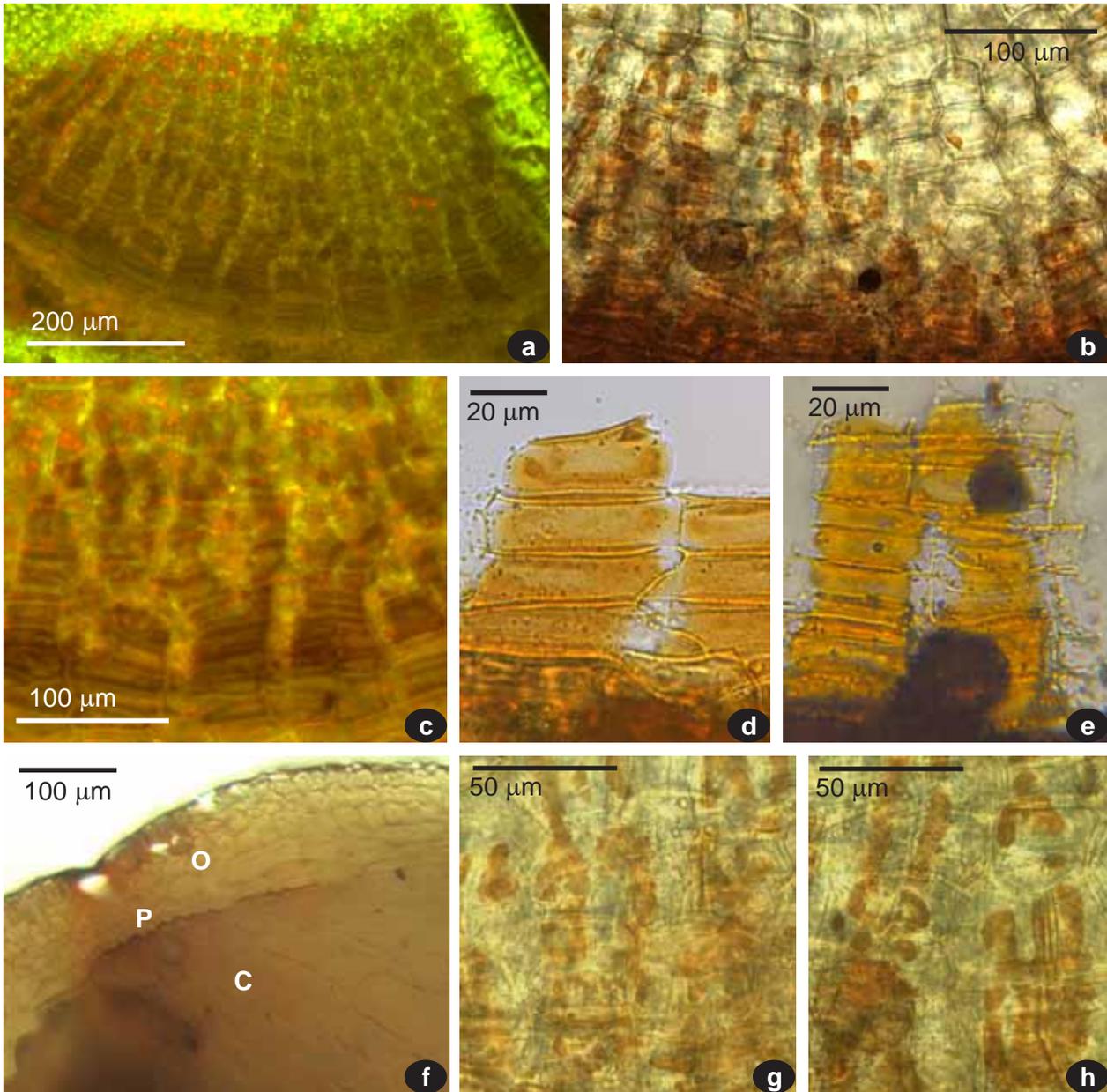


Fig. 7. Peristome of *Scouleria rschewinii* (a-d, f-h – from Vitim River, 10.VII.1984, *Bardunov*, MHA; e – from Ust-Belaya, 19.VIII.1980, *Afonina*, LE): a, c – operculum from inside with outline of peristome, as seen in fluorescent microscope, with berberin staining; b, g, h – taken from the same operculum as a and c in transmitted light: note that peristomial material in very transparent and almost unseen, with only partial secondary deposition of thick brownish material. After capsule opening, most of peristome material remains attached to the operculum, and teeth are usually seen only by their bases (d, e and compare with Fig. 6e); f: longitudinal section showing operculum (O), peristome (P) and columella (C); this peristome is also shown in a-c, g, h.

Fig. 6 (opposite page). Peristome and columella in *Scouleria pulcherrima* (a-b, f, h-k – from Chukotka, 13.VIII.1979, *Afonina*, *Bryophyta Rossica exsiccata* 186, LE; g – from Tungir River, 2000, *Golyakov*, LE) and *S. rschewinii* (c-d – from Vitim River, 10.VII.1984, *Bardunov*, MHA; e – from Irkutsk Province, 18.VII.1984, *Bardunov*, LE). Note that columella has a widening in the former species with a cavity above it (likely due to tissue resorption in proximity of peristome teeth) and adjoining to operculum by narrow band of large-celled tissue. At the same time, poor peristome teeth development in *S. rschewinii* (c-d, and cf. Fig. 7f) results in no such cavity and widening of columella, which adjoins to operculum by much wider zone. Teeth are irregular in shape (b, g-k), but are well developed.

from Baikal Lake area to the left Amur River tributaries.

According to the molecular phylogenetic analysis, the Chara+Olekma populations have the genetic differentiation at about the same level as that between *S. aquatica* and *S. siskiyouensis* in North America. However, lack of morphological differentiation does not allow a serious

discussion of its taxonomic recognition. Some other haplotypes may be recognized in *Scouleria* specimens from Siberia, including one that has smaller cells (blue in Fig. 2), but other plants of the same haplotype have larger cells, thus, at present, these characters are not enough for discussion.

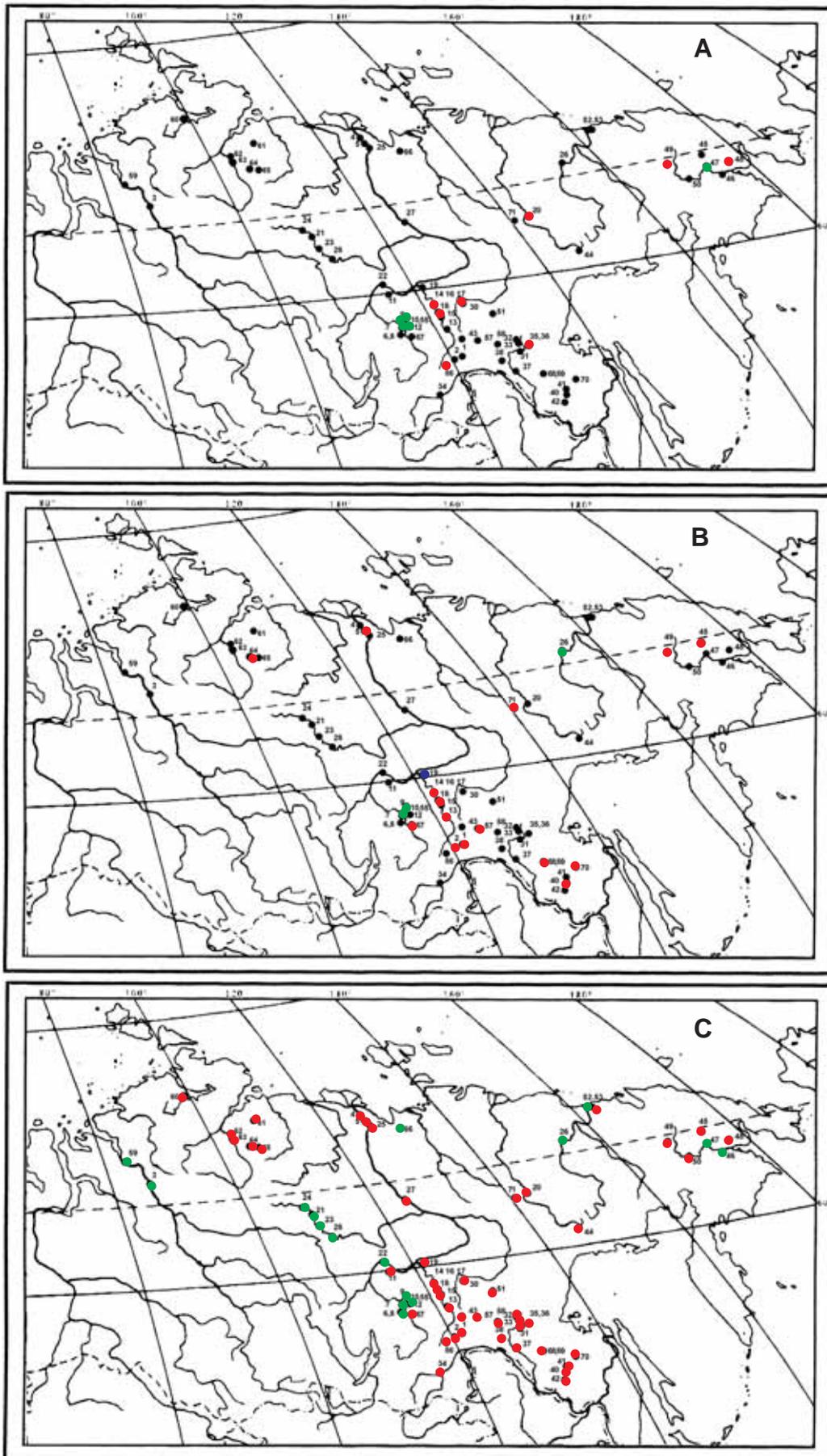


Fig. 8. Distribution of *Scouleria pulcherrima* (red) and *S. rschewinii* (green). Genetically distinct population of *S. pulcherrima* referred to *S. rschewinii* by Carter *et al.* (2014) is marked by blue dot. Numbers of localities correspond with those given in brackets in specimens examined sections.

A – plants with sporophytes;

B – sequenced specimens;

C – known distribution based on gametophytic characters rectified in the course of combined molecular and morphological analysis.

TAXONOMIC TREATMENT

Scouleria Hook., Bot. Misc. 1: 33. 1829[1830].

Type: *Scouleria aquatica* Hook.

Plants aquatic, dark olive-green or blackish-green, often becoming black and glossy when dry, leaves in lower parts of shoots often with lamina partially destroyed by running water. Stems long, irregularly branching, rigid, lacking central strand, with dense rhizoids at base. Leaves crowded, loosely appressed when dry, erect-spreading to spreading when moist, ovate or lingulate, weakly concave, with rounded and often cucullate apex, margins irregularly dentate or bluntly serrate in proximal 2/3–3/4, entire at base; costa strong, slightly bending, ending well below leaf apex, in transverse section with one row of guide cells and 2–3 rows of substereids on dorsal and ventral side; lamina unistratose, in proximal half or 2/3 often with bistratose submarginal strips formed of elongate cells, and/or with thickened unistratose margins formed of several rows of enlarged thicker-walled cells; distal and median laminal cells thick-walled, isodiametric, rounded or rounded-polygonal, areolation dim; basal laminal cells short-rectangular to elongate-rectangular, basal marginal cells subquadrate, several rows of submarginal cells occasionally elongate and bistratose, forming a border. Dioicous. Perigonia lateral, sessile. Archegonia terminal or subterminal, inner perichaetial leaves lingulate or ovate-lanceolate. Sporophytes emergent. Seta short and thick, straight. Capsules immersed or emergent, systylious, globose when young, compressed and becoming ring-like after dehiscence, often 2–3 times shorter than wide, strongly narrowed to the mouth, dark brown, sometimes with rusty tint, or almost black when mature, glossy; exothecial cells moderately thick-walled, stomata absent; columella stout, longitudinally ribbed. Annulus absent. Operculum almost flat or low conic, with low knob-like beak or apiculate, attached to the columella. Peristome of 32 fragile, short, triangular reddish teeth, inserted below the mouth of capsule, trabeculate on outer surface, smooth on inner side, blunt at apices, reflexed when dry, slightly incurved when wet, or peristome teeth somewhat reduced, slender, with distal parts remaining attached to the inner surface of the lid after partial dehiscence. Spores spherical, large, finely granulate. [Calyptra cucullate, smooth and naked].

KEY TO IDENTIFICATION OF *SCOULERIA* SPECIES
IN RUSSIA

1. Leaves with several rows of larger and thicker marginal cells, forming a darker border, and occasionally also in proximal 1/3–1/2(–2/3) of leaf with bistratose submarginal border formed of several rows of elongate cells; peristome teeth not strongly fragile, usually present in deoperculate capsules, reflexed when dry; columella ± narrowly attached to the operculum; operculum with moderate subobtusate beak ..
..... *S. pulcherrima*

— Leaves not bordered, marginal cells not thicker than median laminal cells; peristome teeth strongly fragile, adherent to the inner surface of the operculum; columella wide at place of attachment to the operculum; operculum with very small and acute apiculus ..
..... *S. rschewinii*

1. **Scouleria pulcherrima** Broth. Trudy Bot. Muz. Imp. Akad. Nauk 16: 24. 2 f. 1, 4 f. 2. 1916.

Figs. 9-11; 3c; 4a, c; 5b, d, g; 6 a-b, f, g-k.

Plants dark green distally or black. Stems 5–15 cm long, repeatedly branched, rigid, densely foliate. **Leaves** ± straight or slightly wavy when dry, erect-spreading to spreading when moist and remaining slightly undulate, 2–5×0.8–1.5 mm, ovate-lanceolate or lingulate, concave, widely rounded and cucullate at apex; **margins** irregularly dentate, darker than lamina, marginal border unistratose but thickened, composed of larger cells with thicker walls, and occasionally also with bistratose submarginal strips 1–4 cells wide, formed of 2–4(–5) rows of elongate cells, shining or brownish, forming submarginal border in lower 1/3–1/2(–2/3) of leaf; **costa** strong, 100–120 µm wide at base, in transverse section with one row of guide cells and 2–3 rows of substereids on dorsal and ventral sides, occasionally with rhizoids on dorsal or on both sides at base or up to 1/2 of leaf; upper and median laminal **cells** rounded or rounded-polygonal, 9–24 µm, ± thick-walled, dim, in proximal 2/3 of lamina hardly stained by polarized light, basal laminal cells short rectangular to elongate rectangular, basal marginal cells in 2–5 rows subquadrate, submarginal cells in 2–5 rows elongate, forming a border. Perichaetial leaves with widened base, ca. 3.0×1.0–1.1 mm. **Seta** 0.8–1.0 mm. **Capsules** emergent, dark brown, dark reddish brown or almost black, ca. 0.8–1.5 mm long, 1.3–1.5 mm wide, globose when young, flattened and becoming ring-like after dehiscence. Exothecial cells transversely short rectangular, moderately thick-walled, walls straight. Columella widened at some distance below operculum, then narrowing above this widening and narrowly attached to the operculum. **Peristome** teeth triangular, irregular distally, moderately fragile, usually present in deoperculate capsules, orange-brownish, 200–250 µm long. **Spores** 40–55 µm, finely granulate.

Differentiation. *Scouleria pulcherrima* shares with the very variable North American *S. aquatica* a similarly differentiated marginal border, consisting of larger and thicker-walled cells, and often also bistratose submarginal border of elongate cells. Their morphological differences include some tendency in leaf shape and costa structure: in *S. pulcherrima*, leaves are often lingulate, more widely rounded at apex, and with less stout costa, without differentiated surface layers and with substereids instead of stereid bands, while in *S. aquatica*, leaves are often more abruptly tapered distally, more narrowly rounded at apex and have stronger costa with differenti-

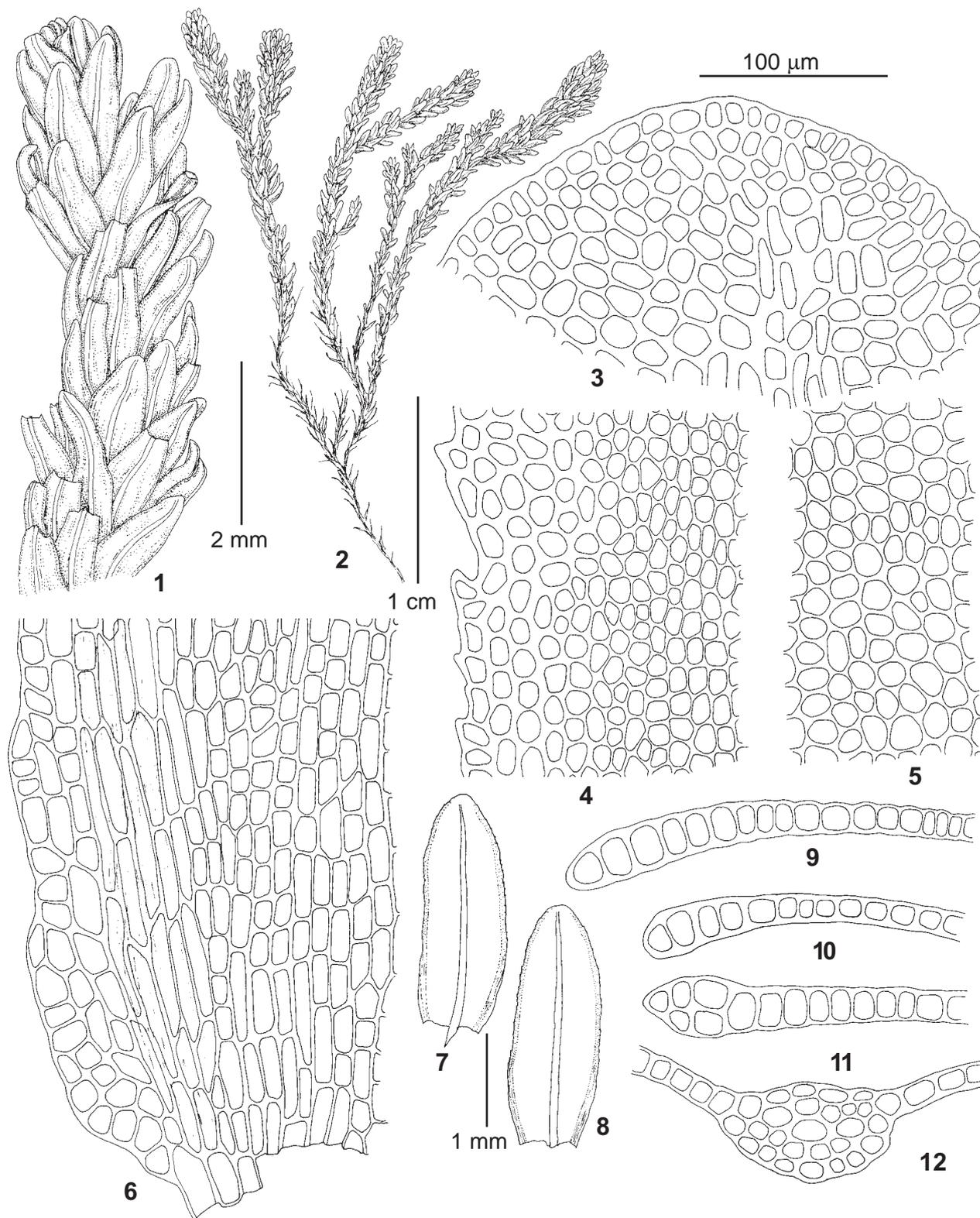
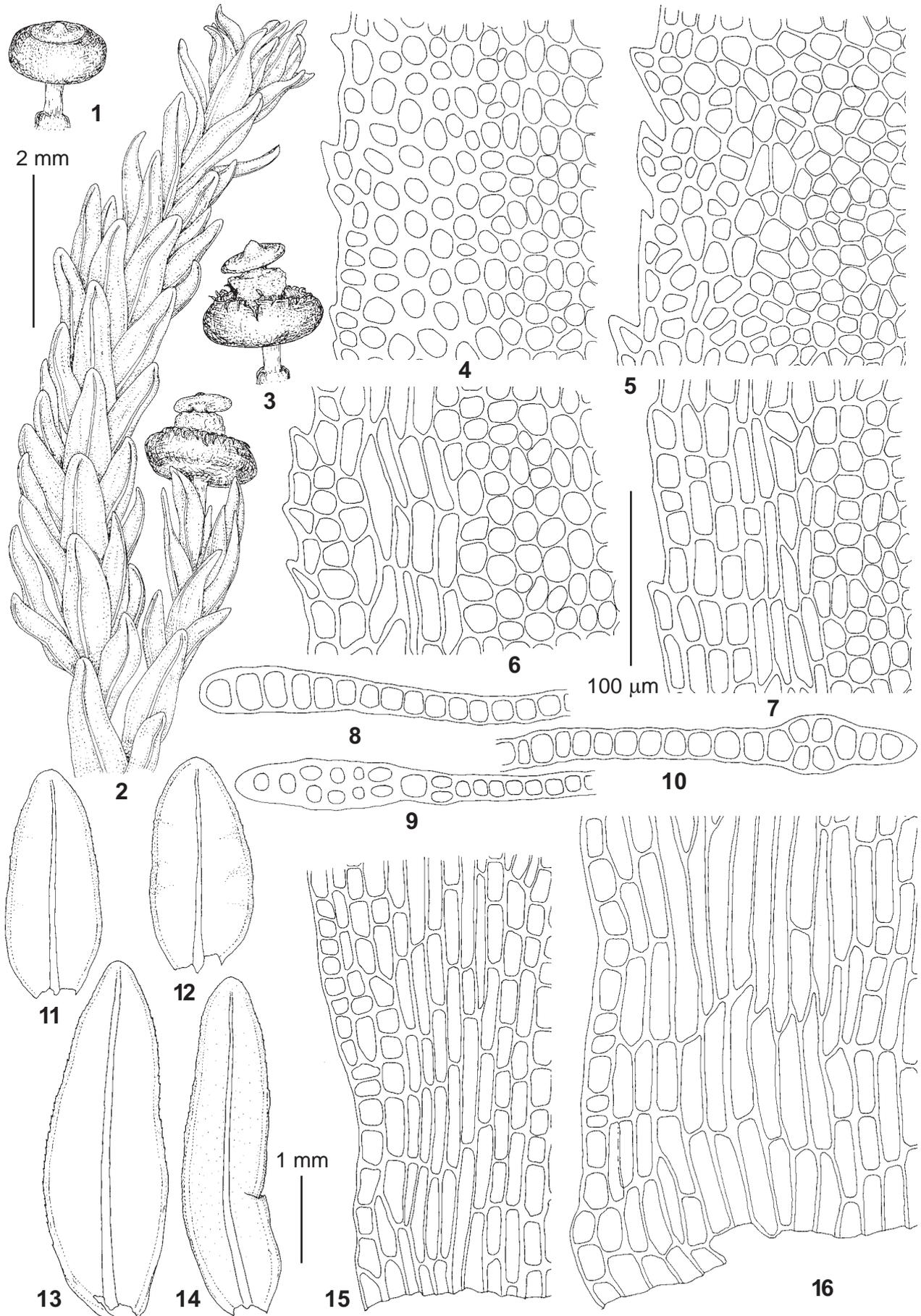


Fig. 9. *Scouleria pulcherrima* Broth. (from lectotype: Regio amurensis, Zei, fl. Siwakan, 19/6 1911, *O. Kuzeneva s.n.*, H-Br 3810002): 1-2 – habit, dry; 3 – upper laminal cells; 4-5 – median laminal cells; 6 – basal laminal cells; 7-8 – leaves; 9-11 – leaf transverse sections at margin; 12 – transverse section of costa. Scale bars: 1 cm for 2; 2 mm for 1; 1 mm for 7-8; 100 μ m for 3-6, 9-12.

Fig. 10 (opposite page). *Scouleria pulcherrima* Broth. (1-3 – from: Russia, Yakutia, Olekma River, 1.VII.1998, *Golyakov s.n.*, LE; 4, 8, 11-12, 16 – from: Russia, Krasnoyarsk Territory, Anabar Plateau, *Fedosov 07-150*, MW; 5, 7, 10, 13, 15 – from: Russia, Amurskaya Province, Selemdzha River, 4.VI.2011, *Bezgodov 171*, MHA; 6, 9, 14 – from: Russia, Magadan Province, Kolyma River, 23.VI.2012, *Mochalova s.n.*, MHA): 1, 3 – capsules; 2 – habit, dry; 4-7 – median laminal cells; 8-10 – leaf transverse sections at margin; 11-14 – leaves; 15-16 – basal laminal cells. Scale bars: 2 mm for 1-3; 1 mm for 11-14; 100 μ m for 4-10, 15-16.



ated ventral and dorsal epidermis and two stereid bands. Differences from *S. rschewinii* are discussed under that species.

Distribution and ecology. *Scouleria pulcherrima* is sporadically distributed in mountain areas of East Siberia, Yakutia, Chukotka, and in the Russian Far East (Khabarovsk Territory and Amurskaya Province). It is rather common and abundant in rivers and creeks of south-eastern Taimyr (Anabar Plateau), Olekma – Chara Upland, Stanovoy Range and Stanovoe Upland, and in the northern part of Verkhoyansky Range (lower course of Lena River); it was also sporadically collected in river systems of Northern Yakutia and Anadyr Plateau in Chukotka. Grows on seasonally submerged cliff bases, boulders and pebble bars on river and creek banks and in water courses, occasionally also on boulders and pebble bars at lake shores.

Specimens examined (numbers in brackets correspond with those on maps in Fig. 8): ASIAN RUSSIA: Lectotype: [Amurskaya Province] Regio amurensis. Zeii, fl. Siwakan, 19.VI.1911, *Kuzeneva s.n.* (H-Br 38100002); Krasnoyarsk Territory: Taimyr: [62] Popigai River at Khayargas-Yuryagh Creek mouth, *Fedosov 08-344* (MHA); [65] Kotujkan River, *Fedosov 07-150, 07-142, 07-227, 07-222* (MHA); [63] Kotuj River 5 km downstream Medvezh'ya River mouth, *Fedosov 05-62* (MW); [64] Kotuj River at Medvezh'ya River mouth, *Fedosov 05-439, 05-570* (MW); [66] Kotujkan River near Merkyu River mouth, *Fedosov 11-692* (MW); [61] Dikson District, Byrranga Mts, Taimyrskoe Lake, Ledyanaya Bay, 10 & 15.VI.2004, *Fedosov s.n.* (MW); Republic of Sakha/Yakutia: [15] Olekma River near Tas-Khaiko Creek mouth, 1.VII.1998, *Golyakov s.n.* (LE); Olekma River, 14 & 22.VIII.1995, *Krivoshapkin s.n.* (LE); [14, 16, 17] Olekma River at Malaya Dzhikimdy Creek, 23.VIII.1995, *Krivoshapkin 02.07.07.12, 02.07.07.13, 02.07.07.16* (MHA); [18] Olekminsky Nature Reserve, middle course of Olekma River, Ytylakh Island, 14.VIII.1995, *Krivoshapkin s.n.* (LE); [19] Olekminsky District, near Byas'-Kuel Settlement, lower course of Chara River, VIII.1996, *Krivoshapkin s.n.* (LE); [13] Olekminsky Nature Reserve, Olyekma River near Khanya Creek mouth, 18.VI.1905, *Golyakov s.n.* (LE); [4] lower course of Lena River, Bulkur, 21.VII.1878, *Nilsson-Ehle s.n.* (LE); [5] lower course of Lena River, Tigije Creek 2 km upstream its mouth, 11.VII.2006, *Pisarenko op01135* (NSK, MHA); Ukhtaa Creek, 16.VII.2006, *Pisarenko y12/02* (NSK); Oimyakon District, vicinity of Uglovoi Settlement, upper course of Indigirka River, 27.VII.2009, *Nikolin s.n.* (SASY, MHA); [20] Oimyakon District, Nera River, near Gusiny Settlement, 23.VII.1960, *Dobretsova 48/1* (LE); [21] Suntar District, Vilyui River 217 km downstream Ulakhan-Vava (near Lokharchaana River mouth), 13.VIII.1958, *Kil'dyushevsky s.n.* (LE); [[25] lower course of Lena River, right bank 15 km downstream Kyusyur Settlement, near Kuramys River mouth, 29.VI.1956, Anonymus (LE); [53] Nizhnekolymsk District, Kolyma River near Petushki Settlement, 28.VIII.1984, *Stepanova s.n.* (LE); [27] Zhigansk District, lower course of Undyulyung River, 70 km downstream Siegemtse Lake, 24.VII.1990, *Stepanova s.n.* (LE); [30] Aldansky District, Uchur River 150 km from Chagda Settlement, Chyl'byu meteorological station, 28.VII.1991, *Ivanova s.n.* (LE); [51] Uchur River 45 km upstream Ili River mouth, 14.VII.1991, *Ivanova s.n.* (LE); Chukotsky Autonomous District: [48] Anadyr River basin, Golubaya River – tributary of Tanyurer River, 13.VIII.1979, *Afonina, Bryophyta Rossica ex-*

siccata 186 (LE, S); [45] Anadyr River basin, Enmyuvaam River, 03.VII.1980, *Afonina s.n.* (LE); [49] Anadyr River basin, Yablun River, 7.VII.1982, *Afonina s.n.* (LE); [50] middle course of Anadyr River, 22.VIII.1982, *Afonina s.n.* (LE); Magadan Province: Yagodninsky District, Kolyma River 3 km upstream Zapyataya Brook, 23.VI.2012, *Mochalova s.n.* (MHA); [44] Seimchen River near Seimchen Settlement, 30.VI.1979, *Khokhryakov s.n.* (LE); Khabarovsk Territory: [40] Bureya River basin, middle course of Tuyun River, 4.VII.1996, *Kurbatova, Bryophyta Rossica exsiccata 184* (LE, MHA, MW); Bureinsky Nature Reserve, Pravaya Bureya River 6 km upstream its confluence with Levaya Bureya River, *Ignatov 97-1088* (MHA); [39, 41, 24] Tyrma River basin, Yaurin Creek 2, 10 and 25 km upstream its mouth, 07.VII.1996, *Kurbatova & Rummyantseva s.n.* (LE); same place, Tyrma River 25 km downstream Yaurin Creek mouth, 06.VII.1996, *Kurbatova & Rummyantseva s.n.* (LE); Amurskaya Province: [31] Zeya River at Tukuringra Range, 31.VII.1914, *Prokhorov & Kuzeneva 188* (LE); [32] left bank of Gilyui River between Nikolaevsky Creek and Inka River, 23.III.1905, *Sokolov 648* (LE); [33] Gilyui River along trail from Morozkin Klyuch to Mogoktak, 04.VIII.1910, *Prokhorov & Kuzeneva 353* (LE); [58] Zeya River basin, Vozdvizhensky mine, Sivakan River, 1911, *Kuzeneva* (LE); [36] unnamed lake near Zeya River upstream of Zhurban Settlement, 07.07.1970, *Vlastov s.n.* (LE) [now this place is covered by the waters of Zeiskoe Reservoir]; [35] Zeya River upstream Algaya River mouth, 23.VI.1970, *Vlastov s.n.* (LE); [38] Zeya District, Amazar River, IX.1971, *Dymina s.n.* (LE); [37] Zeya District, middle course of Zeya River near Rublevka Settlement, VIII.1973, *Gambaryan s.n.* (MHA); Norsky Nature Reserve, Seledzha River, 11 & 17.VI.2011, *Bezgodov 171* (MHA); [43] Tynda District, Olyekma River near Agikta River mouth, 6.VIII.1996, *Golyakov, Bryophyta Rossica exsiccata 185* (LE, MW); [57] Olyekma River near Bes-Yuryakh creek mouth, 2000, *Golyakov s.n.* (LE); Irkutsk Province: [11] Bodaibo District, Bol'shoi Patom River, 08.VIII.1948, *Sheldyakova s.n.* (LE); Zabaikalsky Territory: [34] Sretensk, Shilka River, 14.VIII.1963, *Bardunov s.n.* (LE); [1, 2] Tungir River, 17 & 21.VII.1996, *Golyakov s.n.*; *Bryophyta Rossica exsiccata 183* (LE); [55] Tungir River upstream Tanatra Creek mouth, 2000, *Golyakov s.n.* (LE); [56] Tungir River, Baldyk Creek mouth, 2000, *Golyakov s.n.*; [67] Kalar'skiy District, Stanovoye Nagorje Uplands, Yuzhno-Mujskiy Range, valley of Vitim River, *Mamontov 321* (LE, MHA).

2. *Scouleria rschewinii* Lindb. & Arnell, Kongl. Svenska Vetensk. Acad. Handl., n.s. 23(10): 160. 1890.

Plants olive-green in distal ends of shoots, dark brownish- or blackish green below. Stems 5–10(–15) cm long, repeatedly branched, ± thin, densely foliate. **Leaves** flexuose to contorted when dry, erect-spreading to spreading when moist and remaining slightly undulate, 2.2–3.5(–4) × 0.7–0.8(–0.9) mm, ovate-lanceolate, weakly concave, narrowly rounded at apex, not or slightly cucullate; **margins** bluntly denticulate except the lowermost leaf base, not bordered; **costa** moderately strong, 50–75(–80) µm wide at base, often bending distally, in transverse section with one row of guide cells and 2–3 rows of substereids on dorsal and ventral sides, occasionally with rhizoids on dorsal or on ventral or on both sides up to 1/2 of leaf, rhizoids often only at side surfaces of costa neighboring with lamina; upper and median laminal cells rounded or

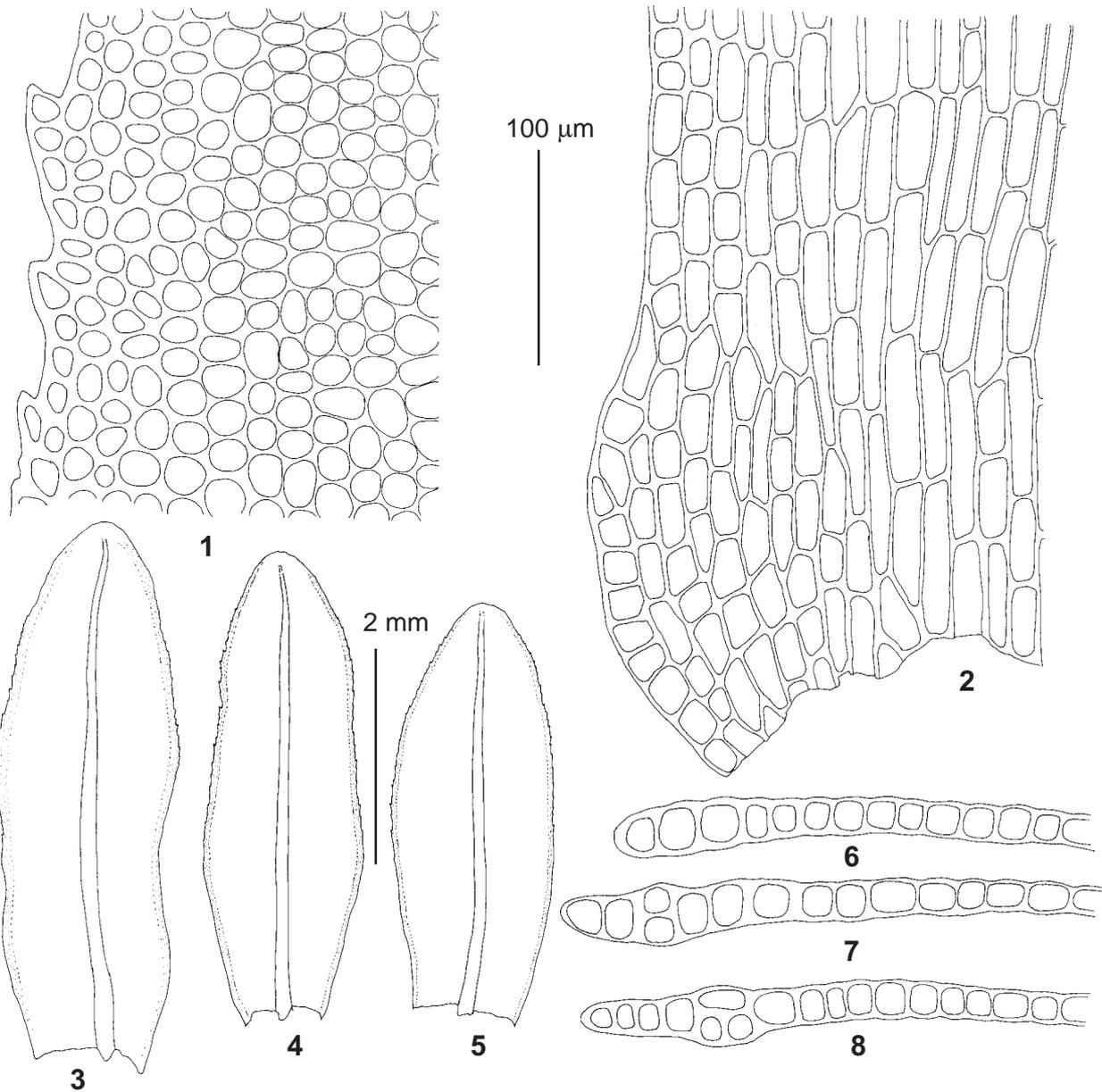


Fig. 11. *Scouleria pulcherrima* Broth. (from: Russia, Irkutsk Province, Chara River, VIII.1996, Krivoschapkin s.n., LE): 1 – median laminal cells; 2 – basal laminal cells; 3–5 – leaves; 6–8 – leaf transverse sections at margin. Scale bars: 2 mm for 3–5; 100 μ m for 1–2, 6–8.

rounded-polygonal, irregular in shape at places, 7–15 (–17) μ m, with moderately thickened walls, \pm translucent, clearly stained by polarized light; basal laminal cells rectangular, 20–50 \times 8–10(–12) μ m, basal marginal cells in 2–5 rows subquadrate. Perichaetial leaves with widened base, ca. 3.0 \times 0.9–1.0 mm. **Seta** ca. 1 mm. **Capsules** immersed to emergent, olive-brown when young, becoming dark brown with age, ca. 0.8–1.0(–1.2) mm long, 1.3–1.8 mm wide, subglobose when very young, flattened and becoming ring-like even when operculum is intact. Exothecial cells transversely rectangular, irregular in shape, moderately thick-walled, cell walls slightly wavy. Columella stout, not narrowing below the operculum. **Peristome** teeth very fragile, with light orange-brown and hyaline-

margined basal portion ca. 30–75 μ m long, remaining at urn mouth in deoperculate capsules, and with faint, light brownish-yellow distal portion being attached to the inner surface of operculum, hardly visible, irregular in shape, ca. 150 μ m long. **Spores** 35–45 μ m, finely granulate.

Differentiation. *Scouleria rschewinii* can be recognized from *S. pulcherrima* by absence of a marginal and submarginal border, either unistratose, consisting of enlarged cells with thicker walls, or bistratose and formed of several rows of elongate submarginal cells. Lamina areolation is more translucent in *S. rschewinii* and cell walls shine in polarized light. Upper and median laminal cells are slightly smaller in *S. rschewinii*, 7–15(–17) μ m vs. 9–24 μ m in *S. pulcherrima*, though their sizes are greatly

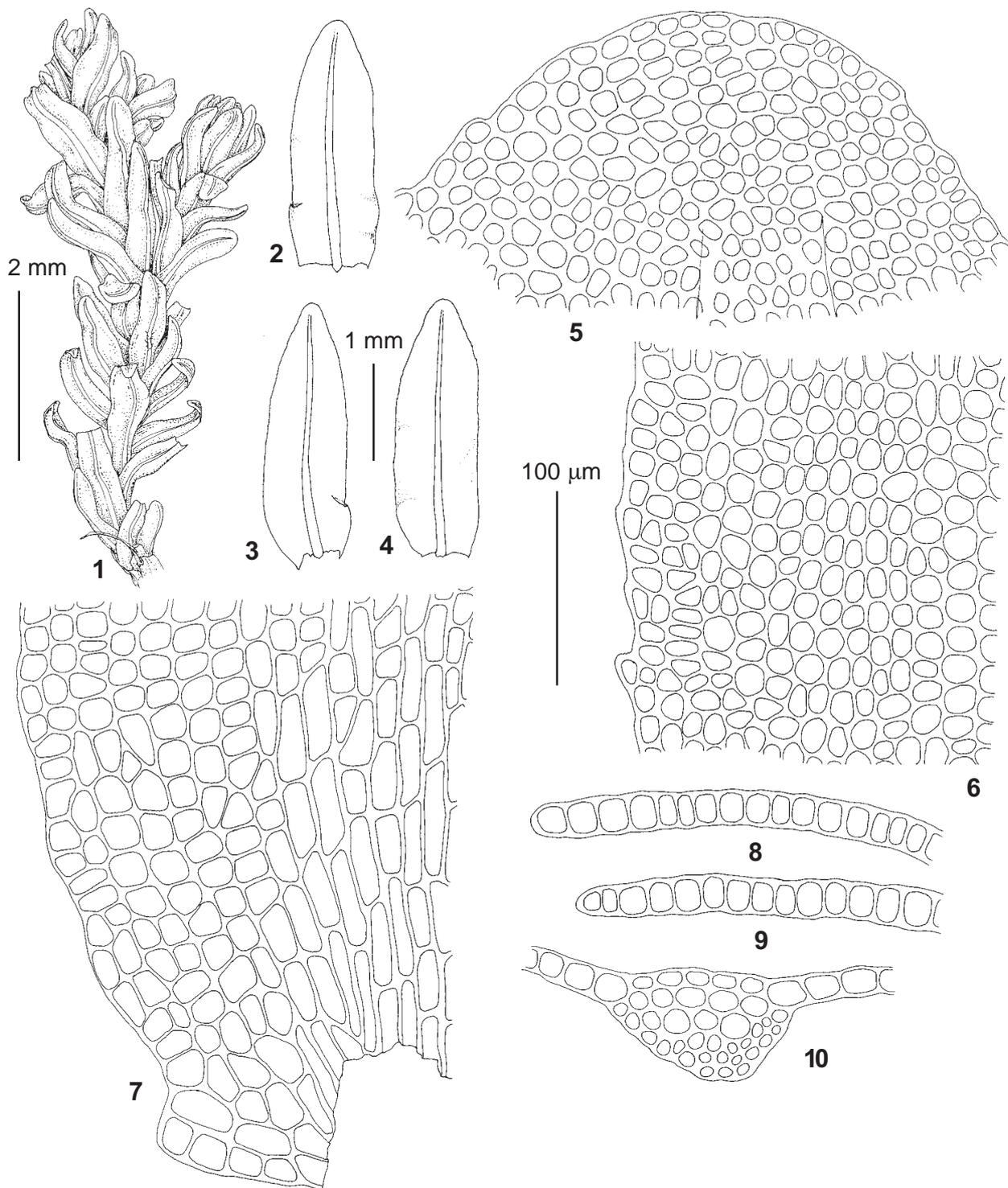


Fig. 12. *Scouleria rschewinii* Lindb. & Arnell (from lectotype: "Siberia, Jenisei, Tolstoinos, 70°10' n. lat., 26/8 1876", *H.W. Arnell*, H-Br 38100014): 1 – habit, dry; 2-4 – leaves; 5 – upper laminal cells; 6 – median laminal cells; 7 – basal laminal cells; 8-9 – leaf transverse sections at margin; 10 – transverse section of costa. Scale bars: 2 mm for 1; 1 mm for 2-4; 100 µm for 5-10.

overlapping. There is a clear difference in columella attachment and peristome structure between these species: in *S. rschewinii*, the columella is uniformly wide and upper portions of peristome teeth are broken off and fall with the operculum being attached to its inner surface, while in *S. pulcherrima*, the columella is narrowed just below the operculum and then widened, forming a welting, and peris-

tome teeth are less fragile, with upper portion intact, not falling off with the operculum.

Distribution. *Scouleria rschewinii* is known from several scattered localities, including the type locality on Yenisey River (the species was never collected on Yenisey since 1876), and also from several distant localities in Yakutia (Vilyui, Kolyma, Omolj Rivers and up-

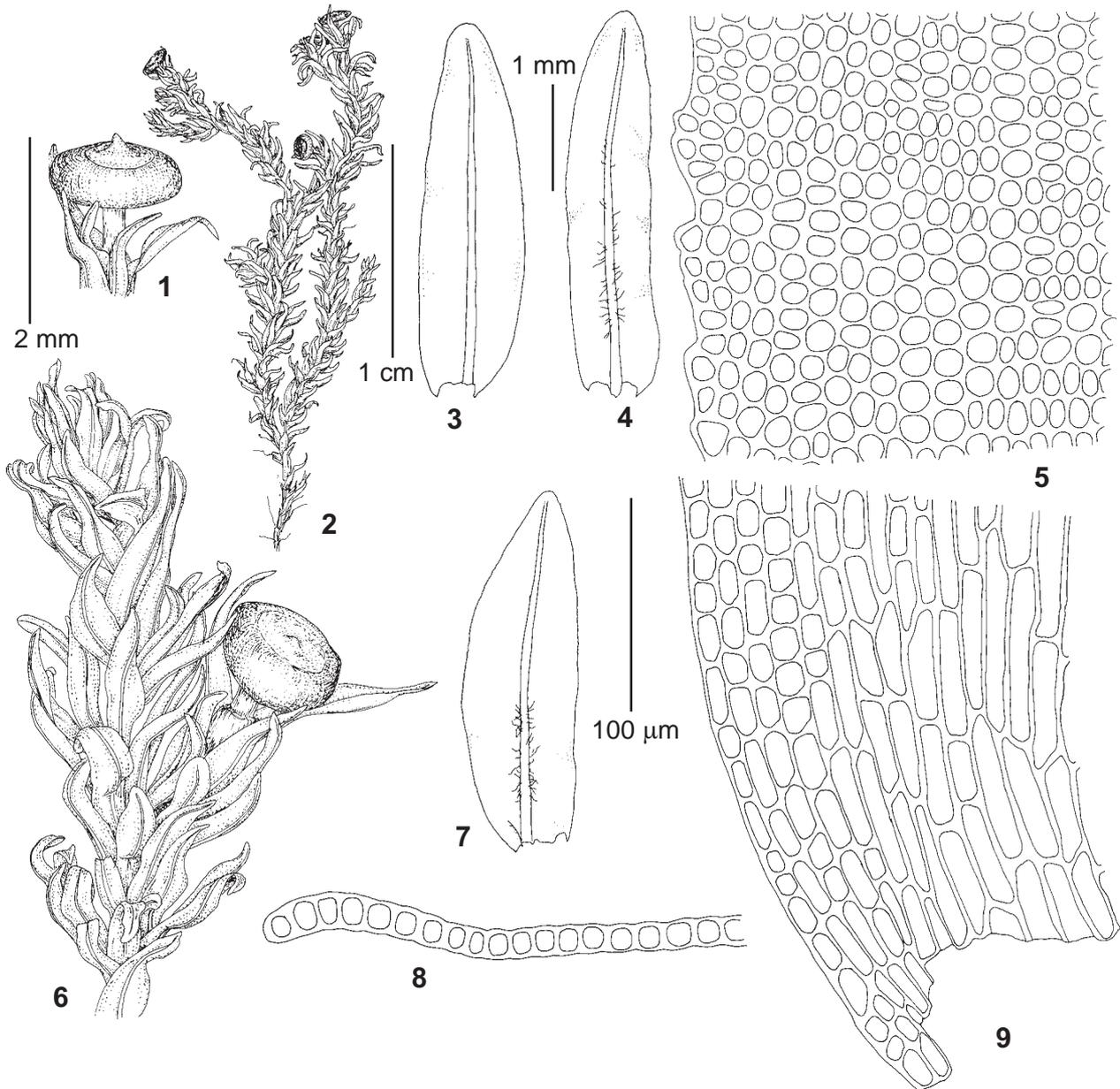


Fig. 13. *Scouleria rschewinii* Lindb. & Arnell (1-2 – from: Russia, Irkutsk Province, Vitimsky Nature Reserve, 10.VII.1984, Bardunov *s.n.*, MHA; 3-10 – from: same place, Mamontov 464/1, LE): 1 – capsule; 2, 6 – habit, dry; 3-4, 7 – leaves; 5 – median laminal cells; 8 – leaf transverse sections at margin; 9 – basal laminal cells. Scale bars: 1 cm for 2; 2 mm for 1, 6; 1 mm for 3-4, 7; 100 µm for 5, 8-10.

per course of Lena River), from two localities in Chukotka (Anadyr River basin), and from the Vitim River in Irkutsk Province (NE of Baikal Lake), where it is locally abundant. The ecology is similar to the previous species.

Specimens examined: Isolectotype: [RUSSIA: **Krasnoyarsk Territory**]: [60] “Jenisei, Tolstoinos, 70°10' n. lat.”, 26.VIII.1876, Arnell *s.n.* (H-Br 3810004); [3] “Jenisei, Igarskoje, 67°20' n. lat.”, 21.VII.1876, H. Wilh. Arnell *s.n.* (S, LE); **Republic of Sakha/Yakutia**: [66] Ust-Yansky District, lower course of Omoloi River, 1800 m downstream Tylaakh Creek mouth, IX.1980, Lapaev *s.n.* (SASY); [52] Nizhnekolymsk District, Kolyma River near Petushki Settlement, 28.VIII.1984, Stepanova 5887 (SASY, LE); [26] Srednekolymsk District, Kolyma River 3 km downstream

Lobuya Settlement, 16.VIII.1973, Stepanova *s.n.* (LE); same place, Kolyma River 12 km downstream Srednekolymsk, 7.X.2002, Ivanova 5883 (SASY); [23] Vilyui River 354 km downstream Ulakhan-Vava (19 km downstream Chirltso Creek), 22.VIII.1958, Kil'dyushevsky 124/1 (SASY, LE); [24] Vilyui River 19 km downstream Ulakhan-Vava (at Potenkata River mouth), 18.VII.1958, Kil'dyushevsky 32/3 (SASY, LE); [29] Vilyui River at Ottonokh rapids, 15.VIII.1958, Usanova 76 (SASY, LE); [28] Mirnyi District, Vilyuiskoe Reservoir near Chernyshevsky (Rybzavod) Settlement, 28.VII.1979, Vasiljeva *s.n.* (LE); [22] Lensk District, Lena River near Mukhtuya Settlement, 6.V.1953, Kuvaev *s.n.* (LE); **Chukotsky Autonomous District**: [47] Anadyr River basin, Belaya River near Ust-Belaya Settlement, 19.VIII.1980, Afonina *s.n.* (LE); [46] central part of

Anadyr River basin, middle course of Utyosiki River, 19.VIII.1948, *Avramchik s.n.* (LE); **Irkutsk Province**: Bodai-bo District, Vitimsky Nature Reserve: [9, 12] Amalyk River near its mouth, 9.VIII.1984, *Bardunov s.n.* (MHA); [6, 8] Verkhny Yuryakh Creek, 12 & 18.VII.1984, *Bardunov s.n.* (LE); [7] Vitim River near mouth of Nizhny Yuryakh Creek, 10.VII.1984, *Bardunov s.n.* (MHA); [10] Oron Lake, 4.VIII.1984, *Bardunov s.n.* (LE); [55] Vitim River, near Amalyk Cordon, *Mamontov 464/1* (LE, MHA).

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LITERATURE CITED

- BROTHERUS, V. F. 1924. Musci (Laubmoose) 1. Hälfte. 10. – In: H. G. A. Engler & K. A. E. Prantl, eds. *Nat. Pflanzenfam.* (ed. 2). *Duncker & Humblot, Berlin*, 478 pp.
- CARTER, B., S. NOSRATINIA, & J.R. SHEVOCK. 2014. A re-visit of species circumscriptions and evolutionary relationships in *Scouleria* (Scouleriaceae). – *Syst. Bot.* **39**: 4–9.
- CHURCHILL, S. P. 1981. A phylogenetic analysis, classification and synopsis of the genera of the Grimmiaceae (Musci). – *Advances Cladist.* **127**–144.
- CHURCHILL, S. 1985. The systematics and biogeography of *Scouleria* Hook. (Musci: Scouleriaceae). – *Lindbergia* **11**: 59–71.
- CHURCHILL, S. 2007. Scouleriaceae. – In: *Flora of North America Editorial Committee* (eds.). *Flora of North America North of Mexico, Vol 27: Bryophyta: Mosses, part 1.* *Oxford University Press, New York, NY*, pp. 311–313.
- FREY, W. 1970. Blattentwicklung bei Laubmoosen. – *Nova Hedwigia* **20**: 463–556.
- GARDINER, A., M. IGNATOV, S. HUTTUNEN & A. TROITSKY. 2005. On resurrection of the families Pseudoleskeaceae Schimp. and Pylaisiaceae Schimp. (Musci, Hypnales). – *Taxon* **54**: 651–663.
- GOFFINET, B. 2009 [2008]. Morphology, anatomy, and classification of the Bryophyta. – In: *Bryoph. Biol. Ed. 2.* *Cambridge University Press, Cambridge, U.K.*, pp. 55–138.
- HALL, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. – *Nucl. Acids. Symp. Ser.* **41**: 95–98.
- HUELSENBECK, J., & F. FRONQUIST. 2001. MRBAYES: Bayesian inference of phylogenetic trees. – *Bioinformatics* **17**: 754–755.
- IVANOV, O.V. & M.S. IGNATOV. 2011. On the leaf cell measurements in mosses. – *Arctoa* **20**: 87–98.
- IVANOV, O.V. & M.S. IGNATOV. 2013. 2D Digitization of plant cell areolation by polarized light microscopy. – *Cell and Tissue Biology* **7**(1): 103–112.
- [KURBATOVA, L.E.] КУРБАТОВА Л.Е. 1998. Род *Scouleria* Hook. в России. – [Genus *Scouleria* Hook. in Russia] *Новости сист. низш. раст.* [Novosti Sist. Nizsh. Rast.] **32**: 162–169.
- LINDBERG, S.O. & H.W. ARNELL. 1890. Musci Asiae Borealis. – *Kongl. Svenska Vetensk.-Akad. Handl.* **23**(10): 1–163.
- NYLANDER, J. A. A. 2004. MrModeltest v2. Program distributed by the author. – *Evolutionary Biology Centre, Uppsala University*.
- [SAVICZ-LYUBITSKAYA, L.I. & Z.N. SMIRNOVA] САВИЧ-ЛЮБИЦКАЯ Л.И., З.Н. СМЕРНОВА. 1970. Определитель листостебельных мхов СССР. Верхоплодные мхи. – [Handbook of mosses of the USSR. The acrocarpous mosses] *Ж., Наука [Leningrad, Nauka]*, 822 pp.
- SCHIMPER, W. P. 1860. Synopsis Muscorum Europeorum. – *E. Schweizerbart, Stuttgart*, 733 pp.
- SCHIMPER, W. P. 1876. Synopsis Muscorum Europeorum. – *E. Schweizerbart, Stuttgart*, 886 pp.
- SHEVOCK, J.R. & D.H. NORRIS. 2014. *Scouleria siskiyouensis* (Scouleriaceae), a new rheophytic moss endemic to southern Oregon, USA. – *Madroño* **61**(1): 137–143.
- STAMATAKIS, A. 2014. RAXML Version 8: A tool for phylogenetic analysis and post-analysis of large phylogenies. – *Bioinformatics* **30**: 1312–1313.

APPENDIX 1.

GenBank accession number are given in the following order: ITS / rpl32.

Scouleria pulcherrima Seledmzha 1 (Russia, Amurskaya Province, 11.VI.2011, *Bezgodov 171*, MHA): KP308292 / KP308287; *S. pulcherrima* Seledmzha 2 (Russia, Amurskaya Province, 17.VI.2011, *Bezgodov 259*, MHA): KP308293 / –; *S. pulcherrima* Vitim 1 (Russia, Irkutsk Province, Vitim, *Mamontov 321/1*, LE): KP308294 / KP308290; *S. pulcherrima* Vitim 2 (Russia, Irkutsk Province, Vitim, *Mamontov 321/1*, LE): KP308295 / KP308288; *S. pulcherrima* Olekma 2 (Russia, Yakutia, Olekma, VII.1996, *Golyakov s.n.*, LE) KP308301 / –; *S. pulcherrima* Bureya (Russia, Khabarovsk Territory, Bureya, *Ignatov 97-1088*, MHA): KP308302 / KP308286; *S. pulcherrima* Tungir (Russia, Zabaikalsky Territory, Tungir River, 21.VII.1996, *Golyakov s.n.*, LE): KP308303 / –; *S. pulcherrima* Chukotka 1 (Russia, Chukotka, 20.VII.1981, *Afonina s.n.*, LE) KP308306 / KP308285; *S. pulcherrima* Chukotka 2 (Russia, Chukotka, 3.VII.1980, *Afonina s.n.*, LE): KP308305 / KP308284; *S. pulcherrima* Chukotka 3 (Russia, Chukotka, 7.VIII.1982, *Afonina s.n.*, LE): KP308304 / KP308289; *S. pulcherrima* var. *Chara* (Russia, Yakutia, Chara River, VIII.1996, *Krivoshapkin s.n.*, LE) KP308299 / –; *Scouleria rschewinii* Vitim 1 (Russia, Irkutsk Province, Vitim, 10.VII.1984, *Bardunov s.n.*, MHA): KP308296 / –; *S. rschewinii* Vitim 2 (Russia, Irkutsk Province, Vitim, *Mamontov 464/1*, LE): KP308297 / KP308291; *S. rschewinii* Vitim 3 (Russia, Irkutsk Province, Vitim, 9.VIII.1984, *Bardunov s.n.*, MHA): KP308298 / –; *S. rschewinii* Kolyma (Russia, Yakutia, Kolyma, 10.VII.2002, *Ivanova* (SASY 5883, MHA): KP342455 / –.