Two new species of Gymnomitriaceae (Marchantiophyta) in the North Pacific

Yury S. Mamontov1,2, Anna A. Vilnet2, Nadezhda A. Konstantinova2 & Vadim A. Bakalin1

ABSTRACT
Two new liverwort species, Gymnomitrion kamchaticum sp. nov. and Marsupella aleutica sp. nov., are described based on integrative taxonomical approach derived from morphology, phytogeography and sequence data. Gymnomitrion kamchaticum is morphologically most similar to Gymnomitrion brevissimum, G. mucronulatum and G. pacificum, but differs from them by leaf insertion, shape of leaves and leaf cells. Marsupella aleutica is morphologically somewhat similar to the Holarctic M. emarginata s.l., M. funckii, M. sprucei, M. ustulata and the East Asian M. minutissima and M. pseudojungermannii, but differs in leaf shape and occasional presence of a tooth at antical leaf base.

Keywords: Hepaticae, taxonomy, new species, Aleutians, Kamchatka, trn L-F cpDNA

The species of the leafy liverwort family Gymnomitriaceae (Marchantiophyta) particularly those of the largest genera of the family, Gymnomitrion Corda and Marsupella Dumort. are mainly restricted to areas difficult to access in upper belts of mountains as well as to polar regions (Väña et al. 2010: 10). As a result the diversity and distribution of species in these genera are still insufficiently known (Väña et al. 2010: fig. 4). The first phylogenetic reconstruction of Gymnomitriaceae by Vilnet et al. (2007) inferred from ITS1-2 nrDNA and trnL-F cpDNA indicates that Gymnomitrion and Marsupella in the traditional meaning are polyphyletic. This study involves analyses of 17 taxa from three of nine genera in this family and is concentrated on the phylogeny of the two largest genera mentioned above. Recently the affinity of genera within the family Gymnomitriaceae were established in molecular-phylogenetic studies of the suborder Jungermanniineae by Vilnet et al. (2010) and Shaw et al. (2015). Recently, in the frame of revision of Gymnomitrion and Marsupella in Europe, Asia and North America efforts are under way to the special estimate of the family diversity under integrative taxonomy approaches. The morphological examination of specimens from the Yunnan Province, China, resulted in the description of a new species Gymnomitrion fiscum Mamontov et Potemkin (Potemkin et al. 2017) and by exploring DNA data the resurrection of Marsupella parvitecta Steph. as a semi-cryptic segregate of Gymnomitrion commutatum (Limpr.) Schiffn. was provided (Mamontov et al. 2018).

The current study aims to describe new species of Gymnomitrion and Marsupella that were found in collections from Kamchatka and the Aleutians during a pilot study of Gymnomitriaceae phylogeny based on integrative taxonomical approach.

MATERIAL AND METHODS
The specimens were examined and photographed using a Leitz Wetzlar Orthoplan light microscope and an Olympus SZX16 stereomicroscope both equipped with digital cameras. The plant habit and some morphological details not visible enough on photo were drawn. The holotype of Gymnomitrion mucronulatum N.Kitag. (8 VIII 1952 D. Shimizu, No. 56660, NICH) was studied to provide its descriptions in Schuster (1974) and Dam-
sholt (2002) were used. The key distinctions of *G. macro-
phorum* R.M. Schust. are provided in the key according to its
description in Schuster (1995). The key for Marsupella spe-
cies is based on the studied type specimens of *M. ateria*,
the specimens of *M. emarginata* s.l., *M. funckii* (F. Weber &
D. Mohr) Dumort. and *M. spurei* (Limp.) Bernet housed in
KPABG, VBGI and MO, and the descriptions of these
species in Schuster (1974) and Damsholt (2002). The key
distinction of *M. minutissima* N. Kitag., *M. pseudofunckii*
S. Hatt and the East Asian taxa of *M. emarginata* s.l. follow
its descriptions in Kitagawa (1963).

**Taxa sampling**

To test phylogenetic affinity of poorly known and presumably
new taxa from the family Gymnomitriaceae the newly
sequenced ITS1-2 nrDNA and *trnL*-F cpDNA loci from 10
specimens of *Gymnomitron* species and a single specimen of
Marsupella from geographically separate regions (China,
Mexico, the Russian Far East and USA) were included to the
dataset from Mamontov et al. (2018). This dataset was
changed by reduction of multiply sampled species and in-
clusion of *Cryptoecopsis imbricata* Amakawa and *Polita
campyloba* Grolle. In total, the alignment produced here
includes nucleotide sequences of 33 taxa and 50 specimens,
where DNA-data for 35 specimens were taken from our
previous studies (Yatsentyuk et al. 2004, Vilnet et al. 2010,
Mamontov et al. 2018) and *trnL*-F nucleotide sequences
were downloaded from GenBank for four *Gymnomitron*
species. The ingroup genera *Gymnomitron* and *Marsupella* are
represented by 26 taxa (including new and morphologically
indeterminate) which is ca 45 % of the worldwide known
species of *Gymnomitron* and *Marsupella* (Söderström et
al. 2016). *Eremotus myriocarpus* (Carrington) Pearson was
chosen as outgroup taxon according to the phylogeny ob-
tained in Vilnet et al. (2010). GenBank accession numbers
and voucher details are listed in Appendix 1.

**DNA isolation, PCR amplification and DNA sequencing**

DNA was extracted from dried liverwort tissue using the
NucleoSpin Plant Kit (Macherey-Nagel, Germany). Amp-
lication and sequencing were performed using primers
given by White et al. (1990) for ITS1-2, Taberlet et al. (1991)
for *trnL*-F. PCR was carried out in 20 µl volumes with the
following amplification cycles: 3 min at 94°C, 30 cycles (30 s
94°C, 40 s 56°C, 60 s 72°C) and 2 min of final extension
time at 72°C. Amplified fragments were visualized on 1 %
agarose TAE gels by EthBr staining, purified using the GFX
DNA and Gel Band Purification Kit (Amersham Bio-
sciences, USA), and then used as a template in sequencing
reactions with the ABI Prism BigDye Terminator Cycle
Sequencing Ready Reaction Kit (Applied Biosystems, USA)
following the standard protocol provided for 3100 Avant
Genetic Analyzer (Applied Biosystems, USA).

**Phylogenetic analyses**

Two nucleotide alignments, ITS1-2 and *trnL*-F, were ob-
tained with the ClustalW option and then manually corrected
with BioEdit 7.0.1 (Hall 1999). The topologies of the strict-
consensus trees of non-parametric bootstrap analyses were
congruent between ITS1-2 and *trnL*-F and consequently
both datasets were combined. All positions of the final
alignment were included in the phylogenetic analysis; parts
of sequences that were lacking and unsequenced loci were
coded as missing.

The combined alignment of ITS1-2+*trnL*-F was analy-
zed using three analytical procedures: the maximum parsi-
mony method (MP) using the TNT 1.5 (Goloboff & Ca-
talano 2016), the maximum likelihood method (ML) using
PhyML v. 3.0 (Guindon et al. 2010) and Bayesian recon-
struction with MrBayes v. 3.2.1 (Ronquist et al. 2012).

The MP analysis involved a New Technology Search with a
search for the minimum-length tree by five reitera-
tions and 1000 bootstrap resamplings; the default set-
ings were used for other parameters, indels were taken
into account by a modified complex coding algorithm in
SeqState (Müller 2005).

The program ModelGenerator (Keane et al. 2004) de-
termined that the GTR+I+Γ model was the best-fit evo-
lutionary model of nucleotide substitutions for the pro-
duced alignment. In the ML analysis the GTR+I+Γ model
was used and the rate of heterogeneity among sites was
modelled using a gamma distribution with four rate cate-
gories. Bootstrap support (BS) for individual nodes was
assessed using a resampling procedure with 500 replicates.

According to the stopping frequency criterion (FC) for
bootstrapping procedure (Pattengale et al. 2010) even 200
replicates were enough for our dataset to reach BS con-
vergence with Pearson average q100 = 0.994324 realized in
RAxML v. 7.2.6 (Stamatakis 2006).

For the Bayesian analysis each partition of the combined
alignment (ITS1-2, *trnL*-F) was separately assigned the
GTR+I+Γ model, gamma distributions were approximated
using four rate categories. Two independent runs of the
Metropolis-coupled MCMC were used to sample parameter
values in proportion to their posterior probability. Each
run included three heated chains and one unheated, and
two starting trees were chosen randomly. Chains were run
for one million generations and trees were sampled every
10th generation. The software tool Tracer (Rambaut &
Drummond 2007) revealed effective sample size (ESS) as
2925.7033 and auto-correlation time (ACT) as 615.2435 for
our data. As determined by Tracer, the first 10000 trees in
each run were discarded as burn-in. Thereafter 90000 trees
were sampled from each run. The average standard devia-
tion of split frequencies between two runs was 0.004825.
Bayesian posterior probabilities were calculated from trees
sampled after burn-in.

The infrageneric and infraspecific variability of each
DNA locus was evaluated as value of p-distances between
specimens and species (Table 1, 2), as calculated in Mega
5.1 (Tamura et al. 2011) using the pairwise deletion option
for counting gaps.

**RESULTS**

**Phylogenetic reconstructions**

ITS1-2 sequences were newly obtained for 11 speci-
mens and *trnL*-F for 10 specimens. The combined align-
ment of the two genomic regions contains 1377 character
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Table 1. The value of infrageneric and infraspecific $p$-distances for the genus Gymnomitrion, n/c – non calculated value due to single specimen only, "- -" – non calculated value due to unsequenced DNA locus.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Infraspecific $p$-distances, ITSI-2/trnL-F, %</th>
<th>Infrageneric $p$-distances, ITSI-2/trnL-F, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>G. commutatum</td>
<td>0.2/0.3</td>
<td></td>
</tr>
<tr>
<td>G. crystallinum</td>
<td>n/c/n/c</td>
<td>2.3/1.0</td>
</tr>
<tr>
<td>G. fiscum</td>
<td>n/c/0</td>
<td>3.5/1.2 3.1/0.6</td>
</tr>
<tr>
<td>Gymnomitrion</td>
<td>0.3/0</td>
<td>4.8/2.0 3.8/1.9 5.1/1.7</td>
</tr>
<tr>
<td>indet.1</td>
<td>0.1/0</td>
<td>3.4/3.7 2.6/3.2 3.3/3.0 3.9/2.6</td>
</tr>
<tr>
<td>G. africatum</td>
<td>-/n/c</td>
<td>-/3.1 -/2.6 -/2.3 -/2.1 -/0.7</td>
</tr>
<tr>
<td>G. revolutum</td>
<td>-/n/c</td>
<td>-/3.4 -/2.9 -/2.7 -/2.0 -/1.4</td>
</tr>
<tr>
<td>G. verrucosum</td>
<td>-/n/c</td>
<td>-/3.5 -/2.9 -/2.7 -/2.2 -/1.9 -/2.7</td>
</tr>
<tr>
<td>G. parvisetus</td>
<td>0.3/0</td>
<td>3.9/3.1 3.2/2.4 4.2/2.4 3.8/2.4 3.1/2.8 -/2.8 -/3.3 -/3.3</td>
</tr>
<tr>
<td>G. confluentes</td>
<td>n/c/n/c</td>
<td>4.9/4.1 3.9/3.5 4.9/2.8 4.3/3.9 3.5/4.4 -/3.8 -/4.5 -/4.5 3.3/3.3</td>
</tr>
<tr>
<td>G. commutatum</td>
<td>0.6/0.5</td>
<td>4.9/3.4 4.6/2.8 5.5/2.6 4.9/3.6 4.0/4.2 -/3.9 -/4.6 -/4.4 3.9/3.3 3.1/3.2</td>
</tr>
<tr>
<td>Gymnomitrion indet.2</td>
<td>n/c/n/c</td>
<td>4.9/3.6 4.4/3.0 4.4/2.8 4.6/3.9 3.3/4.3 -/4.0 -/4.7 -/4.5 3.4/3.5 24/3.5 1.6/1.0</td>
</tr>
<tr>
<td>G. obtusum</td>
<td>n/c/n/c</td>
<td>-/n/c -/n/c -/n/c -/5.1 -/5.8 -/5.4 4.1/4.7 30/4.7 3.8/3.9 29/4.0 4.5/-</td>
</tr>
<tr>
<td>Gymnomitrion indet.2</td>
<td>n/c/n/c</td>
<td>4.9/5.1 4.5/4.7 4.7/4.4 5.0/5.1 4.0/5.3 -/5.1 -/5.8 -/5.4 4.1/4.7 30/4.7 38/3.9 29/4.0 4.5/-</td>
</tr>
<tr>
<td>G. brevissimum</td>
<td>1.1/0.4</td>
<td>4.9/5.1 4.5/4.7 4.7/4.4 5.0/5.1 4.0/5.3 -/5.1 -/5.8 -/5.4 4.1/4.7 30/4.7 38/3.9 29/4.0 4.5/-</td>
</tr>
<tr>
<td>G. pacificum</td>
<td>n/c/0</td>
<td>4.5/4.3 4.2/3.8 4.3/3.6 4.7/4.1 3.8/4.5 -/4.2 -/4.9 -/4.8 3.9/3.7 29/3.4 38/2.8 30/3.1 4.0/- 24/1.9</td>
</tr>
<tr>
<td>G. kamohatsumus</td>
<td>0.2/0.2</td>
<td>4.1/5.0 3.9/4.5 3.9/3.9 4.6/4.5 3.1/5.0 -/4.9 -/5.8 -/5.4 3.6/4.1 26/3.7 37/3.5 27/3.9 4.1/- 24/28 0.9/0.8</td>
</tr>
</tbody>
</table>

Table 2. The value of infrageneric and infraspecific $p$-distances for the genus Marsupella, n/c – non calculated value due to single specimen only.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Infraspecific $p$-distances, ITSI-2/trnL-F, %</th>
<th>Infrageneric $p$-distances, ITSI-2/trnL-F, %</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>M. aquatica M. alpina M. emarginata M. arctica M. boekii M. apiculata M. sphacelata M. bolanderi M. funckii M. distichii</td>
<td></td>
</tr>
<tr>
<td>M. aquatica</td>
<td>0.6/0</td>
<td></td>
</tr>
<tr>
<td>M. alpina</td>
<td>n/c/n/c</td>
<td>1.7/3.4</td>
</tr>
<tr>
<td>M. emarginata</td>
<td>n/c/n/c</td>
<td>1.2/2.4 1.0/3.2</td>
</tr>
<tr>
<td>M. arctica</td>
<td>n/c/n/c</td>
<td>1.8/3.8 1.9/3.7 1.1/2.2</td>
</tr>
<tr>
<td>M. boekii</td>
<td>0.2/0.2</td>
<td>2.5/5.3 2.8/5.2 2.8/4.0 3.0/3.2</td>
</tr>
<tr>
<td>M. apiculata</td>
<td>0/0.2</td>
<td>2.8/6.0 2.8/5.0 2.8/3.5 3.2/3.9 2.7/3.7</td>
</tr>
<tr>
<td>M. sphacelata</td>
<td>0.2/1.5</td>
<td>4.4/6.8 2.2/6.4 3.9/5.3 4.0/5.5 4.5/5.2 3.3/5.0</td>
</tr>
<tr>
<td>M. bolanderi</td>
<td>0/0.2</td>
<td>4.0/7.2 2.4/6.9 3.9/5.8 4.1/5.9 4.3/6.0 2.7/5.5 1.5/1.6</td>
</tr>
<tr>
<td>M. funckii</td>
<td>n/c/n/c</td>
<td>4.6/6.8 3.6/6.2 4.6/4.6 4.6/5.1 5.0/4.7 3.4/4.7 2.4/0.7 22/1.3</td>
</tr>
<tr>
<td>M. distichii</td>
<td>n/c/n/c</td>
<td>5.4/7.4 4.7/7.0 5.1/6.1 5.1/5.9 5.8/4.9 4.1/5.6 3.5/3.1 2.6/3.5 3.5/3.1</td>
</tr>
</tbody>
</table>
sites, among them 897 positions belong to ITS1-2 and 480 – to trnL-F. The number of constant positions in ITS1-2 is 605 (67.45 \%) and in trnL-F is 313 (65.21 \%), variable – 273 (30.43 \%) and 155 (32.29 \%), parsimoniously informative – 170 (18.95 \%) and 98 (20.42 \%), respectively. In the combined ITS1-2 + trnL-F alignment 918 (66.67 \%) positions are constant, 428 (31.08 \%) are variable and 268 (19.46 \%) are parsimoniously informative.

The MP analysis yielded eight equally parsimonious trees with a length of 1471 steps, with CI = 0.636891 and RI = 0.800510 calculated in Mega 5.1. The ML calculation resulted in a single tree, the arithmetic mean of Log likelihood was -6735.201952. Arithmetic means of Log likelihoods in Bayesian analysis for each sampling run were -6598.56 and -6595.61. The tree topologies achieved by the three methods are quite similar and does not contradict the previously published tree (Mamontov et al. 2018). Thus, we provide the ML tree with an indication of bootstrap support values (BS) calculated in the MP and ML analyses and Bayesian posterior probabilities (PP) (Fig. 1).

Three main clades can be distinguished in the tree topologies. The species attributed to the genus Gymnomitron composes the first clade with BS = 100 \% in MP, BS = 74 \% in ML and PP = 1.00 \% in BA (or 100/74/1.00). The second clade unites species from the genus Marsupella (66/62/1.00) and Poeltia campylata, which sister relation to Marsupella is not supported. The third clade includes the sister related Prasanthus suecicus (Gottsche) Lindb. and Cryptolepiastrum umbilicata (68/89/1.00). At the moment the species sampling (45 \% of the worldwide known species) is still insufficient to discuss the phylogeny of Gymnnomitriaceae, but the presented data supports the distinction of some poorly known, recently described taxa.

The Himalayan Marsupella crystallocaulon Grolle (1966), transferred to Apomarsupella R.M. Schust. by Váňa (1999) and then to Gymnomitron by Shaw et al. (2015), is here found related to Gymnomitron commutatum by molecular data (65/86/0.99). Thus, the treatment of this species as a member of Gymnomitron is supported.

The specimen of the recently described Gymnomitron fissing from China (Potemkin et al. 2017) is placed in a sister relation (90/98/0.99) to a Chinese specimen previously published as G. commutatum in Shaw et al. (2015) and separated from the clade with multiple samples of G. commutatum in Mamontov et al. (2018). This clade is related to G. crystallocaulon (Grolle) Váňa, Crand.-Stotl. & Stotler and G. commutatum (79/97/1.00). The trnL-F sequences of both G. fissing specimens does not provide variability, but is distinct from G. crystallocaulon by 0.6 \% and G. commutatum by 1.2 \%, whereas in ITS1-2 by 3.1 \% and 3.5 \% respectively (Table 1). The nucleotide sequence data obtained here once again confirm the description of G. fissing as a distinct species.

Two newly sequenced specimens of Gymnomitron from Kamchatka Peninsula (96/9/1.00) are found in a sister relation (99/98/1.00) to G. pacificum from Alaska and Bering Island (94/78/). Both samples from Kamchatka reveal variability only in ITS1-2 (0.2\%), whereas trnL-F are stable like that in both G. pacificum specimens. The sequence divergences of an unknown taxon and G. pacificum are 0.9 \% in ITS1-2 and 0.8 \% in trnL-F (Table 1). The level of ITS1-2 sequence divergence in the discussed species pair is lower than in G. crystallocaulon–G. fissing–G. commutatum with the same level of p-distances in trnL-F. Taking the morphological differences into account (see below) together with the level of genetic distances, the studied specimens from Kamchatka are distinguished here as a new species – Gymnomitron kamchaticum sp. nov.

The specimens marked on Fig. 1 as “Gymnomitron indet. 1” and “Gymnomitron indet. 2” need special further study.

The specimen of Marsupella from the Aleutian Is, Alaska (UBC) was found in a sister relation (84/53/0.78) to the clade combined by two specimens of M. aquatica (Lindenh.) Schiffl. (98/100/1.00). The p-distance between the Aleutian specimen and M. aquatica is 1.7 \% by ITS1-2 and 3.4 \% by trnL-F. This specimen differs distinctly in appearance from M. aquatica and other species of the genus. The reliable molecular and morphological differences allow us to describe a new species – Marsupella aleutica sp. nov.

**Gymnomitron kamchaticum** Mamontov, Vilnet et Konstant. *sp. nov.* Fig. 2, 3, 6: A, C, E, F

**Description.** Plants yellowish, golden or reddish-brown, forming loose to rather dense mats. Shoots prostrate or ascending, slender, 240–360 \(\mu\)m wide, up to 6.5 mm long, often irregularly branched, with postical-intercalary branches, rarely simple. Rhizoids sparse, colourless, in loose fascicles. Stem rounded, 75–90 \(\mu\)m in diameter, cells in cross section rounded-triangular to rounded-polygonal, almost isodiametric, (8–)10–16–18 \(\mu\)m, the cortical cells almost not different in size from medullar ones, more or less equally thick-walled, the internal cells also equally thick-walled or with large triangular to nodulose thickenings (Fig. 3: Q). Leaves almost transversely or often somewhat obliquely inserted, then succuous, antically secund, in dry plants strongly pressed, making the plants filiform or almost vermiciform, in moist plants not tightly appressed, erect or erect-patent or erect-imbricate, with leaf base divergent from the stem but lobe apices oriented towards the stem; (309–)340–370–395 \(\mu\)m wide, (373–)405–440–464 \(\mu\)m long, usually 1.05–1.31 times as long as wide, ovate or oblong-ovate, concave, widest in lower 1/3–1/4 of the leaf, occasionally with more or less ampliate leaf base (Fig. 2: I, O, 3: H), bilobed for (0.15–)0.18–0.25–0.29 of the length; sinuses acute to rectangular, with concave or convex margins; lobes triangular or ovate-triangular, apiculate or acute, usually ending in two superposed cells, rarely in one cell (Fig. 3: A–I, M, P); the leaf margins normally neither decolorate, nor erose, never reflexed, almost entire in upper 2/3, in lower third sometimes armed with a partial border of radially elongated and slightly protruding, thin- to moderately thick-walled cells (Fig. 3: I, N) or with 1-celled rounded teeth (Fig. 3: K, R). Marginal leaf cells mostly rounded-rectangular or angulate, subquadrate to radially (rarely tangentially) elongated, (7–)8–12–16 \(\mu\)m wide, (9–)10–14–(–)20 \(\mu\)m long, equally thick-walled (Fig. 3: F, P) or with nodulose thickenings (Fig. 3: C–E, I, M); median cells in lobes (6–)10–14–18 \(\mu\)m wide, (9–)12–18–20 \(\mu\)m long, laminar leaf cells (9–)11–17–19 \(\mu\)m wide, (12–)14–19–25 \(\mu\)m long; cell walls with distinct, concave or convex trignonides (Fig. 3: Q) or moderately to distinctly...
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especially in marginal parts) evenly thickened (Fig. 3: F, K, L, O, P). Cuticle smooth or minutely verruculose. Underleaves absent. Dioecious. Androecia spicate, terminal (Fig. 6: C, E, F) becoming intercalary (Fig. 2: H, J, N). Androecial bracts in 4–10 or more pairs, imbricate, 480–640 µm wide, 480–580 µm long, as wide as long or somewhat wider than long, rounded-ovate to subrotundate, in lower third often armed with elongated protruding cells and rounded teeth. Antheridia obovate to subglobose, one per bract, stalk bidirectional. Gynoecial bracts similar to leaves but much larger and more concave, with apiculate lobes. Otherwise unknown.

Holotypus. RUSSIA. Kamchatka Territory, East Kamchatka, Golikavsk Range, Bakening volcano area, close course of Pravaya Kamchatka River (53°56′45″N, 158°01′31″E), 900 m a.s.l., in crevice in gravelly barrens in alpine belt, 4 August 2015, leg. V.A. Bakalin, No. K-44-19-15 (VBGI, isotypes KPABG, MHA).

Paratypus. RUSSIA. Kamchatka Territory, East Kamchatka, Golikavsk Range, Bakening volcano area, upper course of Pravaya Kamchatka River (53°56′27″N, 158°01′31″E), 900 m a.s.l., in crevice in gravelly barrens in alpine belt, 4 August 2015, leg. V.A. Bakalin, No. K-43-10-15 (KPABG, MHA, VBGI).

Etymology. The epithet ‘kamchatciicum’ comes from the Kamchatka Peninsula where the species was collected.

Distribution and ecology. Gymnomitrium kamchatciicum is known from just two collections but it is likely that the species is underrecorded. The habitats in both collecting localities are rocky substrates at the same altitude (the distance between the two localities is less than 500 m). Both collections are made from open places with scattered Pinus pumila (Pall.) Regel clumps and hepatics mats of Cryptocolea imbricata R.M. Schust., Gymnomitrium concinnatum (Lightf.) Corda, G. brevissimum, Pseudolophozia deltiformis (R.M. Schust. & Damsh.) Konstant. & Vilnet, Preissia quadra (Scop.) Nee near.

Marsupella aleutica Mamon-tov, Vilnet, Konstant. et Bakalin sp. nov. Fig. 4, 5, 6: B, D

Description. Plants small, shoots up to 5 mm long and 500–600 µm wide, in gametangia area up to 700 µm, pectinate, olive green to yellow brownish, but the specimen is quite old and the color is probably modified. Stems erect, simple or with few lateral-intercalary branches and subfloral innovations. Stolons not seen. Stem 125 µm in diameter, cortex of one to two layers of relatively large, hyaline, thin- or thick-walled and slightly tangentially elongated cells 15 × 17–22–25 µm, only slightly smaller and thin-walled innermost cells and cells of the medulla that are mixtures of single small to large cells varying from 10 to 15 µm in diameter. Rhizoids few. Leaves distichously arranged, sometimes almost subobovate and ca. 450 µm wide, 450 µm long, but often wider than long (ca. 1.14–1.4 as wide as long) and then ca. 400–450 µm wide, 320–350 µm long, inserted transversely to somewhat obliquely, then succuous, patent or erecto-patent, somewhat conuplicate to canaliculate, with fold antically oriented and forming a "concave" habit of shoots, almost equal in size apart from gymnoecia slightly larger below, slightly to strongly imbricate especially in the gynoecial area, subequally or often unequally...
bilobed, then with larger ventral part, with rounded bases, sometimes with dorsal base (in one to several leaves per shoot) with a lobe-like tooth (Fig. 4: C, D, E, K, M, N: D, J), with a more or less conspicuous border of rather equally thick-walled 1–2(–3) rows of marginal cells (Fig. 5: F–L). Sinuses descending for 0.2–0.25 of leaf length, variable from acute to rounded V-shaped, U-shaped, in some leaves slightly narrowly reflexed at base, lobes broadly triangular, blunt but more often acute, often ending in one or even two celled apiculi, but cells of apiculi are wide, small, ca. 10–13 µm wide, 8–10 µm long. Leaf margins not reflexed. Marginal cells subquadrate, ca. (8–)10–13 µm diagonally, of-

Figure 2 Gymnomitrion kamchaticum Mamontov, Vilnet et Konstant.: A, B, F, G, K, O – leaves; C, D, I, P – leaf margins in the basal third, with cell outlines indicated; E – female plant, dorsal view; H, J, N – male plants, dorsal view; I, M – sterile plants. Scales: 0.1 mm for C, D, I, P; 0.5 mm for A, B, F, G, K, O; 1 mm for E; 1.5 mm for H; 1.2 for J and M; 2 mm for L. All from isotype (MHA)
Two new species of Gymnomitriaceae

ten rounded-rectangular, with longest side mostly oriented perpendicular to leaf margin. Midleaf cells with indistinct or rather distinct, concave to convex, sometimes confluent trigones, rounded-hexagonal in shape, small, in lobes 10–14 µm in diameter, in the middle (10–)13–15(–17) µm wide, 15–17(–20) µm long, at base slightly larger and elongated,
ca. 14–17 µm wide, 17–20(–25) µm long; cuticle smooth, oil bodies not seen. Dioecious. Only female plants were found in the studied specimen. Bracts (Fig. 4: G, O, P) from 550 × 550 µm to 580–680 µm wide and 420–600 µm long, as long as wide or 1.28–1.38 as wide as long, about 0.15–0.2 bifid, with acute and slightly narrowly reflexed at base sinuses;
Two new species of Gymnomitriaceae

lobes slightly unequal, broadly triangular, acute to subacute or obtuse. Perigynium low in unfertilized plants, juvenile perianth much shorter than bracts (Fig. 4: J, P). Otherwise unknown.

Holotypus. USA. Alaska, Shumagin Islands, Simeonof Island (54°55’N 159°15’W), leg. W.B. Schofield, No. 103958 (UBC, sub Marsupella alpina; isotype KPABG, sub Marsupella alpina).
Etymology. The epithet ‘aleutica’ comes from the Aleutian Islands chain (Simeonof Island) where the species was collected.

Distribution and ecology. Currently Marsupella aleutica is known only from the Simeonof Island. According to Schofield et al. (2004) it is “the highly “alpine” species” occurring infrequently “at the higher elevations, where the crowberry heath forms continuous slopes”.

DISCUSSION

Gymnomitrion kamchaticum

Differentiation. Due to erecto-patent (in moist condition) antically secund leaves and often wholly entire leaf margins, the dioecious specimens of Gymnomitrion kamchaticum were initially identified as Marsupella brevissima (Dumort.) Grolle. The latter species is monoecious, but Damsholt
(2002) notes that “pure male and female plants perhaps also occurring”. It should, however, be noted that sterile plants of the new species differ from Marsupella brevissima (= Gymnomitrium brevissimum) in more trapezoidal shape of leaves, mucronate leaf lobes and sometimes crenulate or toothed margins in lower third of leaves. Among other regional taxa, dry plants of Gymnomitrium kamchaticum are most similar to G. pacificum and G. mucronulatum by its filiform habit, and to G. mucrophorum by mucronate leaf lobe apices. Due to suggested occurrence of pure male and female plants in G. brevissimum, an artificial key is provided to distinguish the sterile plants of the discussed five species:

1. Plants usually chestnut-brown or fuscous black. Leaves distally erect-sheathing to erect-imbricate, never tightly appressed, with lobes usually (not when erect) ending by a micro, with margins serrulate or ± clearly crenulate over the whole length or near lobe tips or near leaf base, with marginal cells in 1–3 rows and usually (at least in part of leaves) variously differentiated, that is, thin-walled and slender, or moderately to strongly thick-walled, often elongated obliquely or at right angles to the margin .................................................. G. brevissimum

2. Leaf lobes obtuse to nearly patent, not tightly appressed, with lobes acute or subacute at apex, normally entire-margined, with marginal 1–2 cell rows usually persistent, not differentiated from intramarginal cells, not hyaline at maturity, ± tangentially elongated ........................................ G. pacificum

3. Leaf lobes serrulate with thick-walled, suberect to obliquely inserted and antically secund; the lobes usually slightly elongate cells whose strongly thick-walled lumen, with free ends projecting as rounded protuberant free ends ........................................ G. mucrophorum

4. Leaves in moist shoots erect to nearly patent, not tightly appressed, contiguous or occasionally distant, somewhat obliquely inserted and antically secund; the lobes usually symmetric, often almost triangular and isoseles, divergent with acute to right angle; in upper 2/3 of leaf the leaves entire-margined, in lower 1/3 of leaf the margins entire or sometimes crenulate with rounded protruding cells or 1-celled rounded teeth; the marginal cells with a rounded-multangular or rounded-rectangular lumen, with interior walls slightly to moderately thickened, with concave to convex trigones; in upper 2/3 of leaf the marginal cells similar to the laminar ones in shape and size, in lower 1/3 of leaf the marginal cells partly projected and ± elongated radially, forming at places partial border ........................................................................ G. kamchaticum

Characteristics of localities. The area where Gymnomitrium kamchaticum was collected occupies an intermediate position between the Central Kamchatka with its subcontinental climate and the easternmost fringes of the peninsula with its oceanic climate (cf. Bakalin & Klimova 2018). The site is situated in the way of Pacific wet air masses via Avacha River Valley to the Central Kamchatka depression and the only mountain that may intercept some moisture in wet air masses is the Bakening volcano, thus the area near locus classicus. Both localities are in the transitional zone between alpine and subalpine vegetation. Although the dominant bedrocks are relatively acidic andesite (although less acidic than granite) we may suspect the presence of base-rich volcanic ashes that permit such taxa as Pelinopis quadrata (Saut.) Müll. Frib. and Mesoptychia heterodendrops (Thed. ex Hartm.) L. Söderstr. & Váňa to grow in the crevices near loci classici. These base-rich ash solutions may influence for the habitats where the new taxon was collected.

Marsupella aleutica

Differentiation. Marsupella aleutica differs from all morphologically similar species by the frequent development of a basal tooth on the dorsal margin of sterile leaves. Such a marker is until now unknown in the Holarctic species of the genus (cf. Kitagawa 1963, Schuster 1974, Paton 1999). The species is similar to M. funckii in the leaf orientation, cell size and stem anatomy. It differs from it in color, being olive-green to yellow-brownish vs. deep green to blackish green in M. funckii, much shorter sinuses that are not deeper than 0.2–0.25 of leaf length whereas reaching 0.33–0.45 in M. funckii, even more smaller leaves and cells of margins, thick-walled cells in the middle of leaves, slightly reflexed sinus margin in some leaves, somewhat complicate leaves with uneven lobes, that is not characteristic for M. funckii. The species is similar to species of Marsupella sect. Ustulatae Müll. Frib. ex R.M. Schust. in the size, the shape and orientation of leaves, but differs from it in dioicy, different color of plants being green, yellow-green to light brownish-green versus fuscous to scorched in M. sprucei and M. ustulata Spruce, absence of stolons that are usually numerous in sect. Ustulatae, not largely distally leaved shoots that are characteristic for M. sprucei and M. ustulata. According to obtained molecular results M. aleutica is closely related to M. emarginata (Ehrh.) Dumort. and M. aquatica, the members of the former complex M. emarginata s.l. (cf. Kitagawa 1963, Schuster 1974, Damsholt 2002), which probably includes also North American M. paroica R.M. Schust. and a number of East Asian taxa – M. emarginata sensu Kitagawa (1963). M. aleutica agrees with M. emarginata s.l. by distichously arranged, conduplicate to canaliculate leaves, but differs in much smaller size, much smaller leaf cells, not reflexed margins of leaves and evenly thick-walled marginal cells, also
stem anatomy. From *M. paroica* it also differs by dioicy (vs. paroicy). Among the East Asian taxa, *M. pseudofunckii* and *M. minutissima* are most similar in appearance to the new taxon. *M. pseudofunckii* has asymmetric leaves with distinctly unequal leaf lobes (Kitagawa 1963), whereas *M. aleutica* has more or less symmetric leaves with nearly equal lobes. *M. minutissima* has much longer sinuses, up to 0.33 of leaf length (whereas reaching 0.2–0.25 in *M. alentica*), and leaves longer than wide.

To attempt of generalization the differences between the mentioned above morphologically related species, an artificial key is provided below. This key does not contain all the Holartic *Marsupella* and leaves some species complexes undivided due to priority of determining *M. alentica* and existing of unresolved taxonomic difficulties in need of special studies.

1. Plants large, with sterile parts of shoots up to 0.5–2.5 mm wide, not or slightly broadened in the gyroecium area; leaves often ± reflexed along margins (at least at their bases) ........ 2
2. Plants minute, sterile shoots ca. 300–800 µm wide, leaves much smaller to the base of shoot, gradually larger up the stem, fertile shoots with female bracts much larger than leaves, thus somewhat stoutly (occasionally abruptly) clavate; leaves not reflexed along margins ........................................ 5
3. Leaves asymmetric with distinctly unequal lobes ........................................... *M. emarginata* subsp. *tubulosa* var. *patens*
4. Leaves usually symmetric, with almost equal lobes ........ 3
5. Leaves not reflexed along margins ............................................................ *M. emarginata* subsp. *tubulosa* var. *tubulosa*
6. Leaves reflexed along margins, at least on some upper and subinvolucral leaves ........................................... 4
7. Paroecious ............................................................... *M. paroica*
8. Dioecious ................................................................................. *M. aquatica*, *M. emarginata* subsp. *emarginata* and *M. emarginata* subsp. *tubulosa* var. *apertifolia*
9. Paroecious, almost always fertile; shoots largely distally leaved; leaves moderately to strongly concave, contiguous or weakly imbricate and small near shoot bases, becoming rather closely imbricate and erect-appressed above (especially on fertile shoots), stolons usually numerous .................. sect. *Ustulatae* (M. *spicata* and M. *ustulata*)
10. Dioecious; shoots not so largely distally leaved; leaves patent or erecto-patent or obliquely spreading, ± conduplicate to canalicate; stolons absent or infrequent ............. 6
11. Leaves asymmetric with distinctly unequal lobes ........................................................... *M. pseudofunckii*
12. Leaves usually symmetric with almost equal lobes .......... 7
13. Leaf sinus normally not deeper than 0.25 of leaf length, basal tooth on the dorsal margin of sterile leaves occasional or frequent .................................................. *M. aleutica*
14. Leaf sinus normally reaching 0.33–0.45 of leaf length, basal tooth on the dorsal margin of sterile leaves absent ... 8
15. Leaves longer than wide (ca. 1.14–2.25 as long as wide); ........ .................................................. *M. minutissima*
16. Leaves barely wider than long (ca. 1.03–1.13 as wide as long) ................................................................. *M. funckii*

**Notes on distribution.** Of three specimens referred by Schofield et al. (2004) to *M. alpina* (Gottsche ex Husn.) Bernet we studied one collection and described it as *M. alentica*. The other two collections from Simeonof Island cited by Schofield et al. (2004) should probably also be referred to this species. Schofield et al. (2004) noted that the vegetation of the island was totally destroyed in the course of the last glaciation and was then populated again by wind-blown diaspores starting from 10000 BP or even later. The record of *Marsupella aleutica* may put this suggestion in doubt if this species is found nowhere else. However, taking both the commonness of habitat where *Marsupella aleutica* was collected and the absence of the distinct uniqueness of the island by its nature characteristics into account we may expect the occurrence of this new taxon far beyond the Simeonof Island, and, at least, likely may be observed in the nearest Aleutians or even the Alaska and Kamchatka peninsulas. In general the characteristics of the bryophyte flora of Simeonof Island possesses the dominance of both Arctic and Boreal elements with the latter comprising the overwhelming majority, as in most of the Aleutians.

**CONCLUSION**

Among the studied taxa, *Marsupella aleutica* is most closely related by nucleotide sequences to the Holartic species *M. aquatica*, *M. arctica* and *M. emarginata*. *Gymnomitron kamchaticum* was found in a sister relation to *G. pacificum*, which is known from both sides of the Pacific Ocean – from Alaska, British Columbia and Mexico on the one hand, and from Japan and the Russian Far East (Sakhalin Province, the Kamchatka and Chukotka peninsulas) on the other. According to the phylogenetic relations and rarity of both *Gymnomitron kamchaticum* and *Marsupella aleutica*, are possibly the result of recent speciation in the Northern Pacific under the impact of volcanism as was shown in other liverwort genera (cf. Bakalin & Vilnet 2014). Both species could currently be treated as endemics of Kamchatka Peninsula and Aleutian Islands respectively. Nonetheless, the data on ranges of both species may be extended due to continuation of *Gymnomitron* and *Marsupella* revisions in the North Pacific, including Chukotka, Alaska and the Aleutians.

**ACKNOWLEDGEMENTS**

We are grateful to curators of MO and UBC for lending the collections of Gymnomitriaceae for our taxonomic work. We are greatly indebted to Dr. A. Potemkin and Dr. E. Urmi for helpful comments and criticism. Dr. T. Kata­giri, the curator of NICH herbarium, is kindly thanked for sending the type of *Gymnomitron mucronulatum* for comparative investigation. Mr. A. Hagborg is gratefully acknowledged for fruitful consideration and linguistic correction. The work was partially funded by the Russian Foundation for Basic Research (grants 17-04-00018 and 18-04-00594).

**LITERATURE CITED**


Bakalin, V.A. & K.G. Klimova 2018. Флора печеночников (Нарвиксев) природного парка «Налычево» (поля-
### Appendix 1
The list of taxa, specimens vouchers and GenBank accession numbers, accessions obtained in this study are in bold, accessions downloaded from GenBank are in italic, other – from our previous studies.

<table>
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<th>Taxon</th>
<th>Specimen voucher</th>
<th>GenBank accession number</th>
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