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# TAS3 miR390-dependent loci in non-vascular land plants: Towards a comprehensive reconstruction of the gene evolutionary history

Sergey Y. Morozov<sup>Corresp., 1</sup>, Irina A. Milyutina<sup>1</sup>, Tatiana N. Erokhina<sup>2</sup>, Liudmila V. Ozerova<sup>3</sup>, Alexey V. Troitsky<sup>1</sup>, Andrey G. Solovyev<sup>1,4</sup>

<sup>1</sup> Belozersky Institute of Physico-Chemical Biology, Moscow State University, Moscow, Russia

<sup>2</sup> Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Moscow, Russia

<sup>3</sup> Tsitsin Main Botanical Garden, Russian Academy of Science, Moscow, Russia

<sup>4</sup> Institute of Molecular Medicine, Sechenov First Moscow State Medical University, Moscow, Russia

Corresponding Author: Sergey Y. Morozov  
Email address: morozov@genebee.msu.su

Trans-acting small interfering RNAs (ta-siRNAs) are transcribed from protein non-coding genomic loci and belong to a plant-specific class of endogenous small RNAs. These siRNAs have been found to regulate gene expression in most taxa including seed plants, gymnosperms, ferns and mosses. In this study, bioinformatic and experimental PCR-based approaches were used as tools to analyze TAS3 and TAS6 loci in transcriptomes and genomic DNAs from representatives of evolutionary distant Bryophyta, Marchantiophyta and Anthocerotophyta. We revealed previously undiscovered TAS3 loci in classes Sphagnopsida and Anthocerotopsida, as well as TAS6 loci in Bryophyta classes Tetrarchidiopsida, Polytrichopsida, Andreaeopsida and Takakiopsida. These data further unveil the evolutionary pathway of the miR390-dependent TAS3 loci in land plants. We also identified SGS3-coding sequences in charophytes and hypothesized that the appearance of TAS3-related sequences could take place at a very early step in evolutionary transition from charophyte algae to an earliest common ancestor of land plants.

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## TAS3 miR390-dependent loci in non-vascular land plants: towards a comprehensive reconstruction of the gene evolutionary history

Sergey Y. Morozov<sup>1</sup>, Irina A. Milyutina<sup>1</sup>, Tatiana N. Erokhina<sup>2</sup>, Liudmila V. Ozerova<sup>3</sup>,  
Alexey V. Troitsky<sup>1</sup>, Andrey G. Solovyev<sup>1,4</sup>

<sup>1</sup>Belozersky Institute of Physico-Chemical Biology, Lomonosov Moscow State University, Moscow, Russian Federation

<sup>2</sup>Shemyakin-Ovchinnikov Institute of Bioorganic Chemistry, Russian Academy of Science, Moscow, Russian Federation

<sup>3</sup>Tsitsin Main Botanical Garden, Russian Academy of Science, Moscow, Russian Federation

<sup>4</sup>Institute of Molecular Medicine, Sechenov First Moscow State Medical University, Moscow, Russian Federation

Corresponding author: Sergey Y. Morozov, morozov@genebee.msu.su

**Keywords:** silencing; small interfering RNA; trans-acting RNA; ARF genes; micro RNA; bryophytes; charophyte algae

**Subjects:** bioinformatics, plant science, genomics, molecular biology

**Abbreviations:** dsRNA – double-stranded RNA; miRNA – microRNA; siRNA – small interfering RNA; ssRNA – single-stranded RNA; tasiARF - trans-acting siRNA specific for ARF gene; ta-siRNA - trans-acting siRNA

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**33 ABSTRACT**

34 Trans-acting small interfering RNAs (ta-siRNAs) are transcribed from protein non-coding  
35 genomic loci and belong to a plant-specific class of endogenous small RNAs. These siRNAs  
36 have been found to regulate gene expression in most taxa including seed plants, gymnosperms,  
37 ferns and mosses. In this study, bioinformatic and experimental PCR-based approaches were  
38 used as tools to analyze TAS3 and TAS6 loci in transcriptomes and genomic DNAs from  
39 representatives of evolutionary distant Bryophyta, Marchantiophyta and Anthocerotophyta. We  
40 revealed previously undiscovered TAS3 loci in classes Sphagnopsida and Anthocerotopsida, as  
41 well as TAS6 loci in Bryophyta classes Tetraphidiopsida, Polytrichopsida, Andreaeopsida and  
42 Takakiopsida. These data further unveil the evolutionary pathway of the miR390-dependent  
43 TAS3 loci in land plants. We also identified SGS3-coding sequences in charophytes and  
44 hypothesized that the appearance of TAS3-related sequences could take place at a very early step  
45 in evolutionary transition from charophyte algae to an earliest common ancestor of land plants.

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**49 INTRODUCTION**

50 Plant chromosomal loci of trans-acting small interfering RNAs (ta-siRNAs) and  
51 microRNAs (miRNAs) encode non-protein-coding and protein-coding precursor transcripts,  
52 which are synthesized by RNA polymerase II and include cap-structures and poly-(A) tails. In  
53 plants, primary miRNA transcripts forming internal imperfect hairpins are processed by a protein  
54 complex including DCL1, HYL1 and SERRATE to give RNA duplexes with 2-nucleotide 3'-  
55 overhangs, which are then terminally methylated by specific RNA methylase HEN1. One strand  
56 of such duplexes, being typically of 21 nucleotides in length and representing a mature miRNA,  
57 is selectively recruited to an effector complex targeting a specific RNA for AGO-mediated  
58 edonucleolytic cleavage or translational repression (Rogers and Chen, 2013; Axtell, 2013;  
59 Bologna and Voinnet, 2014; Borges and Martienssen, 2015; Chorostecki et al., 2017).

60 Some specific microRNAs are able to initiate production of ta-siRNAs (more generally  
61 phasiRNAs) by a step-by-step cleavage of long dsRNA precursors representing dicing of the  
62 dsRNA from a defined start point which generates siRNAs in a “phased” pattern. These PHAS  
63 loci include non-coding TAS genes and genes encoding penta-tricopeptide repeat-containing  
64 proteins (PPRs), nucleotide-binding and leucine-rich repeat-containing proteins (NB-LRRs), or  
65 MYB transcription factors (Allen and Howell, 2010; Zhai et al., 2011; Xia et al., 2013; Fei et al.,  
66 2013; Axtell, 2013; Yoshikawa, 2013; Zheng et al., 2015; Komiya, 2017; Liu et al., 2018; Deng  
67 et al., 2018). Biogenesis of ta-siRNAs includes initial AGO-dependent miRNA binding at single  
68 or dual sites of the precursor transcripts and their subsequent cleavage. The further process is  
69 dependent on plant RNA-dependent RNA polymerase 6 (RDR6) and SGS3 proteins participating  
70 in the formation of dsRNA, which is then cleaved in a sequential and phased manner by DCL4  
71 with assistance of DRB4 (dsRNA binding protein). The resulting ta-siRNAs (mostly of 21 bp in  
72 length), similar to miRNAs, are methylated by HEN1 protein (Allen and Howell, 2010; Axtell,  
73 2013; Fei et al., 2013; Yoshikawa, 2013; Bologna and Voinnet, 2014; Komiya, 2017; Deng et al.,  
74 2018).

75 Arabidopsis TAS3a transcript, first identified by Allen et al. (2005), gives rise to two  
76 near-identical 21-nucleotide tasiARFs targeting the mRNAs of some ARF transcription factors  
77 (ARF2, ARF3/ETT and ARF4). Most angiosperm TAS3 primary transcripts are recognized by  
78 miR390 and cleaved by AGO7 at the 3' target site, whereas the 5' miRNA target site is non-  
79 cleavable. However, the number of miR390 cleavage sites, organization of tasiARF sequence  
80 blocks and phasing registers may vary among different TAS3 genes of vascular plants (Allen and  
81 Howell, 2010; Axtell, 2013; Fei et al., 2013; Zheng et al., 2015; Xia et al., 2013; 2017; de  
82 Felippes et al., 2017; Komiya, 2017; Deng et al., 2018). Moreover, miR390 may additionally  
83 target and inhibit protein-coding gene transcripts, such as StCDPK1 related to auxin-responsive  
84 pathway (Santin et al., 2017).

85 Previously, we described a new method for identification of plant TAS3 loci based on  
86 PCR with a pair of oligodeoxyribonucleotide primers mimicking miR390. The method was  
87 found to be efficient for dicotyledonous plants, cycads, conifers, and mosses (Krasnikova et al.,  
88 2009; 2011; 2013; Ozerova et al., 2013). Importantly, at that time the structural and functional  
89 information on bryophyte TAS3 loci was available only for the model plant *Physcomitrella*  
90 *patens* (Arif et al., 2013), and we used our PCR-based approach as a phylogenetic profiling tool

91 to identify relatives of *P. patens* TAS3 loci in 26 additional moss species of class Bryopsida and  
92 several mosses of classes Polytrichopsida, Tetraphidopsida and Andreaeopsida. Moreover, we  
93 found a putative pre-miR390 genomic sequence for an additional moss class, Oedipodipsida  
94 (Krasnikova et al., 2013). Our studies revealed that a representative of Marchantiophyta  
95 (liverwort *Marchantia polymorpha*, class Marchantiopsida) could also encode a candidate  
96 miR390 gene and a potential TAS3-like locus (Krasnikova et al., 2013). This finding extended  
97 the known evolutionary history of TAS3 loci to the proposed most basal land plant lineage  
98 (Ruhfel et al., 2014; Bowman et al., 2017). In addition, we sequenced putative pre-miR390  
99 genomic locus for *Harpanthus flotovianus* (Marchantiophyta, class Jungermanniopsida)  
100 (Krasnikova et al., 2013). Later, our findings of TAS3-like and miR390 loci were experimentally  
101 confirmed in the studies of the transcriptomes of Marchantiophyta plants *M. polymorpha* (Lin et  
102 al., 2016; Tsuzuki et al., 2016) and *Pellia endiviifolia* (class Jungermanniopsida) (Alaba et al.,  
103 2015).

104 New genomic and transcriptomic sequence data for basal Viridiplantae appeared in NCBI  
105 (<http://ncbi.nlm.nih.gov/sra>) and Phytozome (<http://www.phytozome.net>) databases prompted us  
106 to perform new experimental and *in silico* analyses of TAS3 loci in basal taxons of Viridiplantae.  
107 In this paper, we identified previously unrecognized TAS3 loci in classes Sphagnopsida and  
108 Anthocerotopsida, as well as composite TAS6/TAS3 loci in Bryophyta classes Tetraphidiopsida,  
109 Polytrichopsida, Andreaeopsida and Takakiopsida. Additionally, we revealed SGS3-coding  
110 sequences in charophytes and analyzed their evolutionary links.

111

## 112 MATERIALS AND METODS

113 Dried material for *Sphagnum angustifolium* and *S. girgensohnii* were taken from herbarium at  
114 Department of Biology, Moscow State University. Total DNA was extracted from dry plants  
115 using the Nucleospin Plant Extraction Kit (Macherey-Nagel, Germany) according to the protocol  
116 of the manufacturer. For PCR amplification, the following primers were used: a forward primer  
117 Spha-TASP (5'-GGCGRTAWCCYACTGAGCTA-3') and reverse primer Spha-TASM (5'-  
118 TAGCTCAGGAGRGATAMMBMRA-3'). For PCR, 30 cycles were used with a melting  
119 temperature of 94°C – 3', and the next steps are as follows: an annealing temperature 94°C – 20",  
120 65°C – 20", 58°C – 30", and an extending temperature of 72°C followed by a final extension at  
121 72°C for 5'. PCR products were separated by electrophoresis of samples in a 1.5% agarose gel

122 and purified using the Gel Extraction Kit (Qiagen, Germany). For cloning, the PCR-amplified  
123 DNA bands isolated from gel were ligated into pGEM-T (Promega). The resulting clones were  
124 screened by length in 1,5% agarose gel. The plasmids were used as templates in sequencing  
125 reactions with an automated sequencer (Applied Biosystems) 3730 DNA Analyzer with facilities  
126 of “Genom” (Moscow, Russia).

127 Sequences for comparative analysis were retrieved from NCBI  
128 (<http://www.ncbi.nlm.nih.gov/>), Phytozome (<http://www.phytozome.net>) and 1000 Plant  
129 Transcriptome Project (“1KP”) (<http://1kp-project.com/blast.html>). Sequence similarities were  
130 analysed by NCBI Blast at <http://blast.ncbi.nlm.nih.gov/BlastAlign.cgi>. The presence of open  
131 reading frames within  
132 retrieved sequences was analysed at <http://web.expasy.org/translate/>. The nucleic acid sequences  
133 and deduced amino acid sequences were analyzed and assembled using the NCBI. Conserved  
134 domains in the amino acid sequences were identified using the CD-Search of the NCBI.  
135 COBALT, the constraint-based alignment tool for multiple protein sequences  
136 (<http://www.ncbi.nlm.nih.gov/tools/cobalt/>) was used for multiple sequence alignments and  
137 phylogenetic analyses; neighbor-joining tree was obtained with the use of default parameters.  
138

## 139 RESULTS

### 140 TAS3 loci in Bryophyta (classes Sphagnopsida and Takakiopsida)

141 It is commonly accepted that mosses of classes Sphagnopsida and Takakiopsida represent  
142 most basal lineages in Bryophyta (Shaw et al., 2010; 2011; Rosato et al., 2016). Previously,  
143 using primers, which have allowed us to detect pre-miR390 and TAS3 loci in Bryopsida and  
144 some other moss classes, we failed to identify pre-miR390 and TAS3 genes in genus *Sphagnum*.  
145 However, a predicted sequence of pri-miR390 from *Sphagnum fallax* was recently reported (Xia  
146 et al., 2017). This finding prompted us to re-evaluate the occurrence of TAS3-like loci in  
147 Sphagnopsida. To this end, we designed a new pair of degenerated PCR primers Spha-TASP and  
148 Spha-TASM, which differed from those used previously (Krasnikova et al., 2011; 2013). As a  
149 positive control, we used plasmid DNA carrying cloned TAS3 gene of *Andreaea rupestris*, a  
150 representative of basal Bryophyta (Krasnikova et al., 2013). Like the positive control, two total  
151 DNA probes from *Sphagnum angustifolium* and *S. girgensohnii* gave a single main PCR product  
152 of the expected size (Fig. 1). Cloning and sequencing of these PCR fragments revealed two



153 TAS3-like primary structures having 285 (*S. angustifolium*) and 292 (*S. girgensohnii*) bases in  
154 length and exhibiting 96% identity (e-value = 2e-131). We named these loci as Sphan-285 and  
155 Sphgi-292, (Fig. 2, Fig. S1 and Table 1).

156 Peatmosses *S. angustifolium* and *S. girgensohnii* belong to subgenera *Cuspidata* and  
157 *Acutifolia*, respectively (Shaw et al., 2010, 2016). To extend search for TAS3-like loci inside  
158 genus *Sphagnum* we performed bioinformatics analysis of the nucleotide sequences in databases  
159 available at NCBI (Sequence Read Archive) and Phytozome (version 12.1). Phytozome has  
160 recently released genome assembly of bog moss *S. fallax* (version 0.5). Bog moss belongs to  
161 subgenus *Cuspidata* and represents the most closely related moss to *S. angustifolium* (Shaw et  
162 al., 2016). BLASTN search at Phytozome allowed us to reveal a TAS3-like locus (supercontig  
163 super\_37), which has 100% identity to the TAS3 locus of *S. angustifolium* sequenced in this  
164 study (Fig. S1 and Table 1). Unexpectedly, we found an additional TAS3-like locus in *S. fallax*  
165 (transcript Sphfalx0293s0011, supercontig super\_293). This TAS3 locus in bog moss has 277  
166 nucleotides in length and showed only a distant relation to the *S. angustifolium* TAS3 (Fig. 2,  
167 Fig. S1 and Table 1).

168 To further analyze Sphagnopsida TAS3-related loci, we used BLAST analysis of  
169 Sequence Read Archive (SRA), which is the NCBI database collecting sequence data obtained  
170 by the use of next generation sequence (NGS) technology. Assembly of sequence reads of *S.*  
171 *recurvum* (subgenus *Cuspidata*) retrieved by BLAST search using *S. fallax* sequences as queries  
172 revealed two TAS3 loci (Table 1). The first locus (Sphre-283) is 283 nucleotides in length and  
173 has 98% identity to Sphan-285. The second locus (Sphre-277) shows 98% identity to  
174 Sphfalx0293s0011 (Table 1, Fig. S1). These findings indicate that two distant TAS3 loci in  
175 species of a particular subgenus of genus *Sphagnum* are extremely similar.

176 We also analyzed the SRA database of subgenus *Sphagnum* (Shaw et al., 2010, 2016). It  
177 was found that *S. magellanicum* belonging to this subgenus also encode two TAS3 loci called  
178 Sphma-285 (285 nt size) and Sphma-286 (286 nt size) (Fig. S1 and Table 1). Unlike *S. fallax* and  
179 *S. recurvum*, in *S. magellanicum* TAS3 loci are more similar, showing 86% identity (Fig. 2).  
180 Both Sphma-285 and Sphma-286 had 85% identity to Sphan-285 (Fig. 2). It was found that  
181 TAS3-like locus (Sphpa) from one more representative of subgenus *Sphagnum* (*S. palustre*)  
182 exhibited 98% identity to Sphma-285 (Fig. S1 and Table 1). The SRA database also contained  
183 sequence reads of two representatives from subgenus *Subsecunda* (Shaw et al., 2010, 2016). Our



184 BLAST analysis and subsequent assembly of retrieved reads revealed a single TAS3 locus in *S.*  
185 *cribrosum* (Sphcri, 291 nt size) showing 95% identity to Sphan-285 and 81% identity to Sphma-  
186 286 (Fig. 2, Fig. S1 and Table 1) and a partial TAS3-like sequence in *S. lescurii* (Fig. S1 and  
187 Table 1).

188 Analysis of the SRA database of *Takakia lepidozoides* (class Takakiopsida) allowed us  
189 to reveal only one TAS3-like sequence (Takle-207) (Fig. S1 and Table 1). The same sequence  
190 was revealed in a longer assembly which was found recently upon search of 1KP database (Xia  
191 et al., 2017).

192

### 193 **Comparison of sequence organization between TAS3 loci in Bryophyta**

194 Since Takakiopsida and Sphagnopsida are most basal sister lines to all other Bryophyta  
195 (Shaw et al., 2010, 2011; Rosato et al., 2016), it was very interesting to compare the structural  
196 organization of Takakiopsida and Sphagnopsida TAS3 loci with other classes of Bryophyta. Our  
197 previous detailed analysis of approximately 40 TAS3 loci in Bryophyta (Krasnikova et al., 2011;  
198 2013) showed that the general structure of moss TAS3 is similar in all taxa and fits the structural  
199 organization of *Physcomitrella patens* genes, comprising dual miR390 target sites on the 5' and  
200 3' borders and internal monomeric tasiAP2 sequence followed by tasiARF sequence positioned  
201 in 20-30 bases. We revealed that phylogenetic tree of TAS3-like loci in Bryophyta showed clear  
202 subdivision of their sequences into two main clades (see Fig. 5 in Krasnikova et al., 2013). The  
203 first group was formed by a cluster of sequences close to *P. patens* TAS3 species PpTAS3a,  
204 PpTAS3d, and PpTAS3f, and the second one – by those close to PpTAS3b, PpTAS3c, and  
205 PpTAS3e. The recent paper on the structure of TAS3 loci in lower land plants (Xia et al., 2017)  
206 has shown the structure-functional basis for this phylogenetic subdivision. TAS3 species of the  
207 first group (PpTAS3a/PpTAS3d/PpTAS3f cluster) were shown to form class III of TAS3-like  
208 loci and contain, in addition to the previously reported tasiAP2 and tasiARF-a2 sequences, newly  
209 discovered tasiARF-a3 sequence positioned 5' according to tasiAP2. Among TAS3 species of  
210 basal Bryophyta, *Andreaea rupestris* locus 13-Aru (Krasnikova et al., 2013) belongs to class III  
211 (Fig. 3). Two other *A. rupestris* TAS3 loci, 14-Aru and WOGB\_2010369, belong to the  
212 PpTAS3b/PpTAS3c/PpTAS3e cluster which represents TAS3 class II containing only tasiAP2  
213 and tasiARF-a2 sequences (Xia et al., 2017) (Fig. 4). The mentioned above tasiARF sequences,  
214 tasiARF-a2 and tasiARF-a3, showed no sequence similarity suggesting their independent origins.

215 These tasiRNAs were found to be formed from different strands of the TAS3 dsRNA  
216 intermediate and target different regions of ARF genes (Xia et al., 2017). Inhibition of  
217 production of both tasiARF RNAs in *P. patens* resulted in obvious developmental defects  
218 exhibited, in particular, as alterations in gametophore initiation, protonemal branch determinacy  
219 and caulonemal differentiation (Plavskin et al., 2016).

220 Comparison of nucleotide sequences between TAS3 species of several moss classes  
221 revealed in many plants obvious similarity of nucleotide sequence blocks including tasiAP2 site  
222 and immediate upstream 21 bp block occurring in the same 21-bp-phase (Fig. 5). We  
223 hypothesized that this sequence block may correspond to novel previously unrecognized ta-  
224 siRNA in many moss species. Moreover, we found that this hypothetical ta-siRNA might be  
225 cleaved from TAS3, and its minus-strand is complementary to uncharacterized well-conserved,  
226 protein-coding moss mRNA (Fig. S2).

227 BLAST comparison of *T. lepidozoides* TAS3 with known Bryopsida loci showed that  
228 Takle-207 (see above) belongs to class II of TAS3 with typical positioning of tasiAP2 and  
229 tasiARF-a2 sequences (Fig. 6 and Fig. S1). On the other hand, none of Sphagnopsida TAS3-like  
230 sequences (Table 1) showed conventional internal structural organization of the most moss TAS3  
231 species. The only recognizable conserved site, except miR390-targeting regions, was identified  
232 as tasiARF-a2 sequence, which was found to be conserved between two very distant TAS3 loci  
233 in *S. fallax* and *S. recurvum* (Fig. 2).

234

### 235 **TAS3 loci in Anthocerotophyta**

236 Taking into account the finding of TAS3-like loci in classes Sphagnopsida and  
237 Takakiopsida and previously published data (Krasnikova et al., 2013; Xia et al., 2017), one can  
238 conclude that the only remaining blind-spot in land plants with respect to TAS3 is represented by  
239 phylum Anthocerotophyta. Relationships between liverworts, mosses and hornworts are still  
240 obscure. Moreover, the question remains which bryophyte phylum is a sister line to all other land  
241 plants (Qiu, 2008; Shaw et al., 2011; Harrison, 2017). Recent analysis, in which three bryophyte  
242 lineages were resolved, revealed that a clade with mosses and liverworts could form a sister  
243 group to the tracheophytes, whereas the hornworts is sister line to all other land plants (Wickett  
244 et al., 2014). However, analyses of the plastid genome sequences suggested another branching  
245 order of the phylogenetic tree, with hornworts rather than moss/liverwort clade being a sister

246 group to tracheophytes (Lewis et al., 1997; Samigullin et al., 2002; Ruhfel et al., 2014; Lemieux  
247 et al., 2016). Moreover, some very recent nuclear gene comparisons also suggested that  
248 liverworts might be closer to a common ancestor of land plants, and hornworts could be a sister  
249 clade to tracheophytes (Rosato et al., 2016; Bowman et al., 2017).

250 Analysis of the SRA database of Anthocerotophyta revealed a TAS3-like sequence in  
251 *Folioceros fuciformis* (family *Anthocerotaceae*). Unexpectedly, the discovered TAS3-like  
252 sequence (Folfu) was found to be 244 nucleotides in length and obviously similar to Bryophyta  
253 class III TAS3 species (Fig. 7, Fig. S3 and Table 2). The identity of Folfu to some moss TAS3  
254 sequences exceeds 80% being therefore even higher than between some related Bryopsida  
255 species (Fig. 3). Thus these data clearly indicate a close relation of TAS3 in Anthocerotophyta to  
256 Bryophyta TAS3 (excepting Sphagnopsida).

257

### 258 **TAS3 loci in Marchantiophyta**

259 Some of the recent molecular phylogenetic reconstructions suggested that  
260 Marchantiophyta species could represent a sister clade to all other land plants (see above).  
261 Therefore, finding and comparative analyses of TAS3 loci in this taxon represented a significant  
262 interest for understanding early events in TAS3 evolution. In contrast to class Marchantiopsida,  
263 where putative TAS3 and pre-miR390 loci were previously identified (Krasnikova et al., 2013;  
264 Lin et al., 2016; Tsuzuki et al., 2016), for class Jungermanniopsida only potential pre-miR390  
265 loci were found in *Pellia endiviifolia* and *Harpanthus flotovianus* (Krasnikova et al., 2013; Alaba  
266 et al., 2015). Assuming that miR390 was found to be among eight most conserved miRNA  
267 species in land plants (Xia et al., 2013; You et al., 2017; Liu et al., 2018), Jungermanniopsida  
268 could be expected to encode TAS3 loci.

269 To detect new potential TAS3 loci, we performed BLAST analysis of the SRA database  
270 for species of class Jungermanniopsida using *Marchantia polymorpha* TAS3 sequence (1-Mpo)  
271 as a query. Using this approach we revealed a set of reads and assembled a single TAS3-like  
272 locus (Pelen-192) for *Pellia endiviifolia* (192 nt size). In addition, TAS3 locus of 226 nucleotides  
273 in length was found in *Metzgeria crassipilis* (Metc-226) (Fig. 8, Table 2, Fig. S3). The latter  
274 locus was also recently revealed in a search of 1KP database (Xia et al., 2017).

275 TAS3 1-Mpo sequence was further used for BLAST analysis of other Marchantiopsida  
276 sequences available at the NCBI SRA database. As a result, we retrieved sequence reads and

277 assembled five full-length TAS3-like sequences in *Plagiochasma appendiculatum* (Plaap-247),  
278 *Dumortiera hirsuta* (Dumhi-243), *Marchantia emarginata* (Marem-262), *Ricciocarpos natans*  
279 (*Ricna*-235) and *Conocephalum japonicum* (Conja-252) (Fig. 8, Table 2, Fig. S3). Recent  
280 bioinformatics analysis of 1KP database revealed three additional full-length TAS3-like  
281 sequences in *Conocephalum conicum*, *Lunularia cruciata* and *Marchantia paleacea* (Xia et al.,  
282 2017) (Table 2). Thus, totally 11 TAS3-like loci have been found in Marchantiophyta.

283 Comparative sequence analysis showed that structural organizations of Marchantiopsida  
284 and Jungermanniopsida TAS3 loci were quite similar, whereas Marchantiophyta species were  
285 obviously different from those of Bryophyta. These TAS3 species were found to contain two  
286 conserved sequence blocks presumably corresponding to functional ta-siRNAs. One of these  
287 blocks was found in the vicinity of the 3'-terminal miR390 binding site and corresponded to  
288 Bryopsida tasi-AP2 sequence (Krasnikova et al. 2013), whereas another one (tasiARF-a1),  
289 unique among lower land plants, was located closer to the 5'-terminal miR390 binding site in  
290 Marchantiopsida and Jungermanniopsida TAS3 (Tsuzuki et al., 2016; Xia et al., 2017) (Fig. 8,  
291 Fig. S3).

292

### 293 **TAS6 loci in Bryophyta**

294 Previous studies of *P. patens* revealed three novel non-coding PHAS loci (TAS6) which  
295 were located in rather close genomic proximity to PpTAS3 loci (PpTAS3a, PpTAS3d, and  
296 PpTAS3f) and expressed as common RNA precursors with these TAS3 species (Cho et al., 2012;  
297 Arif et al., 2012, 2013). Moreover, miR529 and miR156 were suggested to influence  
298 accumulation of ta-siRNAs specific not only for TAS6, but also for PpTAS3a (Cho et al., 2012).  
299 We have found that localization of TAS6 loci close to TAS3 genes in common transcripts was  
300 not unique for *P. patens* (subclass Funariidae), since these loci were also found to be encoded by  
301 three other mosses of subclasses Bryidae and Dicranidae (Krasnikova et al., 2013).

302 For further search of the combined TAS6/TAS3 loci, we performed bioinformatics  
303 analysis of 1KP database. Although nucleotide sequences of miR156 and related miR529, as  
304 well as their recognition sites in RNA transcripts, are highly conserved among land plants  
305 (Morea et al., 2016; Axtell & Meyers, 2018), the internal sequences between dual  
306 miR156/miR529 recognition sites show little or no similarity even between different TAS6 loci  
307 of *P. patens* (Arif et al., 2012). So we used, as queries for BLAST search, the individual full-

308 length TAS6/TAS3 loci including most characterized locus encoding PpTAS3a (Fig. 9), as well  
309 as those for PpTAS3d and PpTAS3f. First, it was found that in addition to four previously found  
310 Bryopsida species, encoding TAS6/TAS3 loci, these loci could be revealed in basal subclasses  
311 Timmiidae (*Timmia austriaca*) and Diphysciidae (*Diphyscium foliosum*) (Shaw et al., 2011)  
312 (Table 3, Fig. S4). List of TAS6/TAS3 loci in other moss subclasses was also significantly  
313 extended: we found 18 new loci in Bryidae, seven loci in Dicranidae and four loci in Funariidae  
314 (Table 3, Fig. S4). These novel loci showed recognizable but varying sequence similarities to the  
315 PpTAS3a-containing locus (Fig. 9). Second, most importantly, putative TAS6/TAS3 loci were  
316 revealed in 4 basal classes of Bryophyta, namely, Tetraphidiopsida, Polytrichopsida,  
317 Andreaeopsida and Takakiopsida (Table 3, Fig. S4). These novel loci had a similar organization  
318 to Bryopsida TAS6/TAS3 species (Fig. 9). However, no TAS6-specific sequence signatures were  
319 found in the vicinity of genomic *S. fallax* and *M. polymorpha* TAS3 loci upon analysis of the  
320 corresponding Phytozome genome contigs.

321

### 322 **Phylogeny of SGS3 as a characteristic molecular component of TAS3 pathway**

323 It was shown that some species green algae could encode ancient types of dicer-like  
324 proteins, RDRs, and AGOs. On the other hand, no encoded SGS3 proteins were revealed for  
325 these algae (Zheng et al., 2015). Since SGS3 was found to be essential for production of tasiARF  
326 RNAs in moss *P. patens* (Plavskin et al., 2016), we performed sequence to identify possible  
327 SGS3 genes in charophytes. For identification of SGS3 protein orthologs among land  
328 nonvascular plants and charophytes, we used as a query the most conserved region of *P. patens*  
329 SGS3 including short zinc binding zf-XS domain and RNA recognition XS domain (Bateman,  
330 2002; Zhang & Trudeau, 2008). Importantly, the short N-terminal zf-XS domain is characteristic  
331 for functional SGS3 proteins, since the XS domain-containing protein of *Selaginella*  
332 *moellendofii* lacking TAS-generating machinery (Banks et al., 2011) possesses no zf-XS domain  
333 upstream of XS domain and instead contains the C-terminal RING zf region (see NCBI  
334 accession XP\_002979112). However, it should be noted that the lack of TAS3 pathway and  
335 SGS3 is not universal for lycophytes (Xia et al., 2017).

336 In addition to class Bryopsida, SGS3 protein sequences were revealed for members of  
337 classes Marchantiopsida, Jungermanniopsida, Anthocerotopsida, Takakiopsida and  
338 Sphagnopsida (Fig. 10 and Fig. S5). Most importantly, search for the SGS3 coding sequences in

339 transcriptomes of four charophyte classes (*Zygnemophyceae*, *Coleochaetophyceae*,  
340 *Charophyceae*, and *Klebsormidiophyceae*) also revealed the SGS3-like proteins in  
341 representatives of all these taxa (Fig. 10, Fig. S5, Fig. S6). This observation was in agreement  
342 with the fact that SGS3-like coding sequence was found in the fully sequenced and annotated  
343 genome of *Klebsormidium nitens* (NCBI accession GAQ92898) (Hori et al., 2014). Moreover,  
344 the characteristic motifs of land plant SGS3 proteins (Bateman, 2002) were revealed in the  
345 protein sequences from charophyte algae (Fig. S5, Fig. S6).

346 Importantly, in the dendrogram based on comparisons of 24 aligned SGS3 protein  
347 sequences, the position of charophytes (Fig. 10) corresponded to the commonly accepted  
348 Viridiplantae phylogenetic tree (Shaw et al., 2011; Delwiche & Cooper, 2015; Harrison, 2017),  
349 where class *Zygnemophyceae* (*Spirogyra pratensis*) was a sister group for all land plants.  
350 Bryophytes represent the first branching lineage in a land plant subtree of SGS3 proteins, where  
351 ferns and Gymnosperms are clustered as the separate monophyletic groups (Fig. 10). It has  
352 become clear that evolving the SGS3-like genes was not directly connected to the appearance of  
353 TAS loci in Viridiplantae, since Chlorophyta species, lacking SGS3, encode not only critical  
354 enzyme machinery including DCLs, RDRs, and AGOs (You et al., 2017), but also PHAS loci  
355 (Zheng et al., 2015). Despite our extensive searches, no SGS3 genes could be identified also in  
356 brown and red algae, and this is in agreement with previously published data on green algae  
357 (Zheng et al., 2015).

## 358 359 **DISCUSSION**

360 It was proposed that the earliest function of TAS3 could contribute to the production of  
361 ta-siRNAs targeting ARF genes, and, since green algae encode no ARF genes, TAS3 likely  
362 appeared first in land plants (Xia et al., 2017). However, very recent extensive comparative  
363 sequence analysis showed that charophyte algae representing the sister group to all land plants  
364 (colonized terrestrial environments approximately 480 million years ago) could encode ARF-like  
365 proteins including all sequence domains typical for bryophyte and angiosperm ARFs (Mutte et  
366 al., 2017). Moreover, our current data showed that TAS3-like loci are encoded by the  
367 representatives of all main taxa among non-vascular plants. These observations suggest that the  
368 TAS3 evolution started in a common ancestor of land plants, likely belonging to a still unknown  
369 lineage of charophytes. Identification of the canonical motifs of land plant SGS3 in charophyte



370 proteins (see above) indirectly supports this speculation. However, it should be kept in mind that  
371 evolving the SGS3-like genes could not be connected solely to the appearance of PHAS loci in  
372 Viridiplantae, since green algae and brown algae species were found to encode not only essential  
373 silencing machinery enzymes including DCLs, RDRs and AGOs, but also PHAS loci (Billoud et  
374 al., 2014; Zheng et al., 2015; Singh et al., 2015; Zhang et al., 2016; Dueck et al., 2016; You et  
375 al., 2017; Cock et al., 2017). Finally, it can be proposed that the failure to identify charophyte  
376 TAS3 loci may be related to (i) the incompleteness of the available sequence data; (ii) evolving  
377 by charophytes the one-hit TAS3 genes (de Felippes et al., 2017); or (iii) the use of miRNA  
378 species with sequences other than land plant miR390 for TAS precursor processing.

379

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387

### 388 **Author Contributions**

389 Sergey Y. Morozov conceived and designed the experiments, analyzed the data, prepared figures  
390 and/or tables, wrote the paper, reviewed drafts of the paper.

391 Irina A. Milyutina conceived and designed the experiments, performed the experiments,  
392 contributed reagents/materials/analysis tools.

393 Tatiana N. Erokhina and Lydmila V. Ozerova performed the experiments, contributed  
394 reagents/materials/analysis tools, prepared figures and/or tables, reviewed drafts of the paper.

395 Alexey V. Troitsky and Andrey G. Solovyev conceived the experiments, analyzed the data,  
396 wrote the paper, reviewed drafts of the paper.

397

### 398 **DNA Deposition**

399 The following information was supplied regarding the deposition of DNA sequences: The new  
400 sequences generated for this study are available as a nexus file in the Supplemental Material. All



401 sequences used in this study are available on GeneBank (new sequences accession numbers  
402 MF682529 and MF682530).

403

404

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606 **FIGURE LEGENDS**

607

608 **Figure 1:** Analysis of PCR products in 1.5% agarose gel. Amplification of genomic DNA  
609 sequences flanked by miR390 and miR390\* sites. PCR products were obtained on genomic  
610 DNAs with degenerate primers. *Sphagnum angustifolium* (1), *Sphagnum girgensohnii* (2),  
611 *Andreaea rupestris* (3). (M), DNA size markers including bands ranging from 100 bp to 1000 bp  
612 with 100 bp step (Sibenzyme).

613

614 **Figure 2:** Pairwise sequence comparisons of some available nucleotide sequences of TAS3-like  
615 loci from mosses of genus *Sphagnum*. BLASTN was used at NCBI blast site. The miR390 target  
616 sites are in yellow, and putative tasiARF-a2 site is in green.

617

618 **Figure 3:** Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-  
619 like loci from mosses with class III TAS3 locus 13-Aru of *Andreaea rupestris*. BLASTN was  
620 used at NCBI blast site. The miR390 target sites are in yellow; putative tasiARF-a2 site is in  
621 green; tasiAP2 is in blue, and tasiARF-a3 is shaded.

622

623 **Figure 4:** Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-  
624 like loci from mosses with class II TAS3 loci 14-Aru (A) and WOGB\_2010369 (B) of *Andreaea*  
625 *rupestris*. BLASTN was used at NCBI blast site. The miR390 target sites are in yellow; putative  
626 tasiARF-a2 site is in green; tasiAP2 is in blue.

627

628 **Figure 5:** Multiple sequence alignments of nucleotide sequence blocks including tasiAP2 site  
629 and preceding 21 bp site of putative ta-siRNA of *Andreaea rupestris* TAS3-like locus  
630 WOGB\_2010369. BLASTN was used at 1KP blast site. For the complete TAS3 transcript  
631 sequences see Xia et al., 2017. The putative tasiAP2 site is in blue, and preceding putative ta-  
632 siRNA site is in violet. *Andreaea1 - Andreaea rupestris* WOGB\_2010369; *Andreaea2 -*  
633 *Andreaea rupestris* WOGB\_2002765; *Tetraphis1 - Tetraphis pellucida* HVBQ\_2019753;  
634 *Tetraphis2 - Tetraphis pellucida* HVBQ\_2011866; *Tetraphis3 - Tetraphis pellucida* HVBQ  
635 \_2005644; *Plagiomnium - Plagiomnium insigne* BGXB\_2010105; *Leucobryum - Leucobryum*  
636 *glaucum* RGKI\_2062694; *Racomitrium - Racomitrium varium* RDOO\_2117129; *Philonotis -*  
637 *Philonotis fontana* ORKS\_2058791; *Dicranum - Dicranum scoparium* NGTD\_2078536;

638 *Encalypta* - *Encalypta streptocarpa* KEFD\_2058811; *Ceratodon* - *Ceratodon purpureus*  
639 FFPD\_2044193; *Niphotrichum* - *Niphotrichum elongatum* ABCD\_2000143; *Funaria* - *Funaria*  
640 sp. XWHK\_2042016; *Schwetschkeop* - *Schwetschkeopsis fabronia* IGUH 2166854;  
641 *Aulacomnium* - *Aulacomnium heterostichum* WNGH\_2088134; *Syntrichia* - *Syntrichia princeps*  
642 GRKU\_2074985; *Diphyscium1* - *Diphyscium foliosum* AWOI\_2069791; *Diphyscium2* -  
643 *Diphyscium foliosum* AWOI\_2006305; *Hypnum* - *Hypnum subimponens* LNSF\_2068452;  
644 *Pohlia* - *Pohlia nutans* GACA01023180; *Bryum* - *Bryum argenteum* GCZP01053768.

645

646 **Figure 6:** Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-  
647 like loci from mosses with TAS3 of *Takakia lepidozoides*. BLASTN was used at NCBI blast  
648 site. The miR390 target sites are in yellow; putative tasiARF-a2 site is in green; tasiAP2 is in  
649 blue.

650

651 **Figure 7:** Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-  
652 like loci from mosses with TAS3 of hornwort *Folioceros fuciformis*. BLASTN was used at  
653 NCBI blast site. The miR390 target sites are in yellow; putative tasiARF-a2 site is in green;  
654 tasiAP2 is in blue; tasiARF-a3 is shaded.

655

656 **Figure 8:** Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-  
657 like loci from non-vascular plants with TAS3 of *Marchantia polymorpha*. BLASTN was used at  
658 NCBI blast site. The miR390 target sites are in yellow; putative tasiARF-a1 site is in brown;  
659 tasiAP2 is in blue.

660

661 **Figure 9:** Pairwise sequence comparisons of selected nucleotide sequences of TAS6/TAS3-like  
662 loci from mosses with TAS6/TAS3 of *Physcomitrella patens* precursor RNA (accession  
663 JN674513). BLASTN was used at 1KP blast site. The miR390 target sites are in yellow; putative  
664 miR156/miR529 sites are underlined; tasiAP2 is in blue; putative tasiARF-a2 site is in green;  
665 tasiARF-a3 is shaded.

666

667 **Figure 10:** The phylogenetic tree based on sequence alignment of the conserved region of SGS3  
668 amino acid sequences in selected lower plant species. Neighbor-joining tree was obtained at

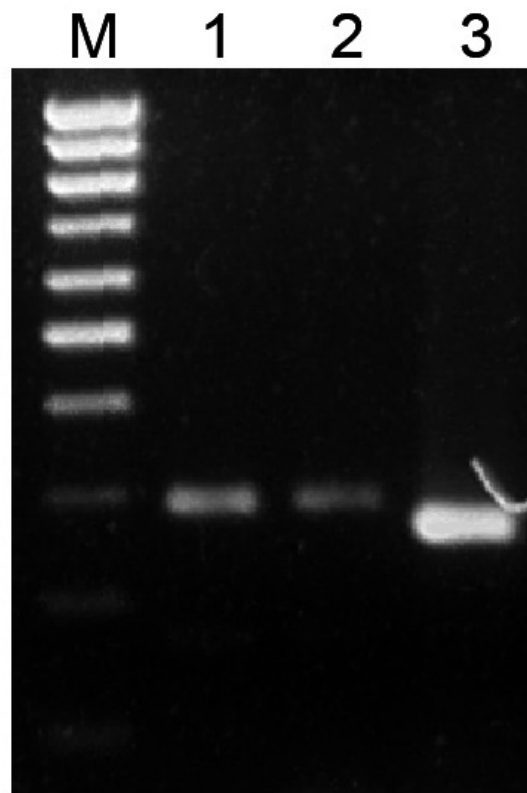


669 <http://www.ncbi.nlm.nih.gov/tools/cobalt/> with the use of default parameters. The scale bar  
670 denotes the estimated number of amino acid substitutions per site. *K. flaccidum* was used as  
671 outgroup.

# Figure 1

Analysis of PCR products in 1.5% agarose gel.

Amplification of genomic DNA sequences flanked by miR390 and miR390\* sites. PCR products were obtained on genomic DNAs with degenerate primers. *Sphagnum angustifolium* (1), *Sphagnum girgensohnii* (2), *Andreaea rupestris* (3). (M), DNA size markers including bands ranging from 100 bp to 1000 bp with 100 bp step (Sibenzyme).



**Figure 2**(on next page)

Pairwise sequence comparisons of some available nucleotide sequences of TAS3-like loci from mosses of genus *Sphagnum*.

BLASTN was used at NCBI blast site. The miR390 target sites are in yellow, and putative tasiARF-a2 site is in green.





**Figure 3**(on next page)

Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-like loci from mosses with class III TAS3 locus 13-Aru of *Andreaea rupestris*.

BLASTN was used at NCBI blast site. The miR390 target sites are in yellow; putative tasiARF-a2 site is in green; tasiAP2 is in blue, and tasiARF-a3 is shaded.



***Physcomitrella patens* cluster TAS3a (accession BK005825) (E-value: 3e-34)**

```

Query 1  GGCGGTAACCCCTTCTGAGCTAAG 23
          ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 1  GGCGTTATCCCTCTTGAGCTGAG 23

Query 69  CATCTTGTAGGCAAGGTGTTAAGCACTTTAGTGCGAGAC-CCTGCCACAAGACGCTAGCT 127
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 70  CACCTTGTITAGCGGGGTGTTAAGCATTTGAATGCAACACTCCT-ACGCAAGACCCTAGCT 128

Query 128  ACAGCTCCC TAGGGTGTGATGAGTGCTTTAGCTGGCACTCATCCACTACCCA GCCCACCT 187
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 129  ATGGCTCCA TAGGGTGTGATGAGTGCTTCA TCCGGTGTCTTCTACTGCCTT GCCCACCT 188

Query 188  ACCCTTGGGACAA GGGCTGTGTACCTCTGCGCAGTC-CCTG--TCGGT TTGTATATCAC 244
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 189  ACCCTTGTGATAI GGGCCGCGC-GTGTCTGCG-TGTCTCTGTATCGG- TTGTATATCAC 245

Query 245  TCCTGAGCTA 254
          ||| ||| ||| ||| ||| ||| |||
Sbjct 246  TCCTGAGCTA 255
          ||| ||| ||| ||| ||| ||| |||

```

***Tetraphis pellucida* clone 80-Tpe (accession KC812753) (E-value: 1e-40)**

```

Query 1  GGCGGTAACCCCTTCTGAGCTAAGTAAGCTGGGGGTGGGTGGAGCCAAGTAGAGGAGG-TT 59
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 1  GGCGGTAACCCCTTCTGAGCTAAGTAGCCAAGGGTTAGCTGTAGGGCAGTAG--GAGCCTT 58

Query 60  TGTAGTGCGCATCTTGTAGGCAAGGTGTTAAGCACTTTAGTGCGAGACCCTGCCACAAGA 119
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 59  TGCCGGGGG--TCTTGTAGGCGGGGTGTTAAGCACTTCGCGTGGGCTCTCCGTGGTAAGA 116

Query 120  CGCTAGCTACAGCTCCCTAGGGTGTGATGAGTGCTTTAGCTGGCACTCATCCACTACCCA 179
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 117  CGTCAGCTATGGCTCCGTAGGGTGTGATGAGTGCTTTA CCTGGCGCTCAACAACCTCCCA 176

Query 180  GCCCACCTACCCTTGGGACAA GGGC--TGTGCTACCTCTGCGCAGTCCTGTCCGGT TTGT 237
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 177  GCCCACCTACCCTTGGGACAC GGGCCAAGTG-GATTTCCACTCGGCCTGTGTCCG- TTGT 234

Query 238  ATATCACTCCTGAGCTA 254
          ||| ||| ||| ||| ||| ||| |||
Sbjct 235  CTATCACTCCTGAGCTA 251
          ||| ||| ||| ||| ||| ||| |||

```

***Encalypta rhytocarpha* clone 31-Erh (accession KC791769) (E-value: 1e-31)**

```

Query 1  GGCGGTAACCCCTTCTGAGCTAAGTAAGCTGGGGGTGGGTGGAGCCAAGTAGAGGAGGTTT 60
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 1  GGCGGTAACCCCTTCTGAGCTAGAAAAG-TAGGCAAGG-----GCCCCCTCGGGGACGAAA 54

Query 61  GTA-GTGCGCA----TCTTGTAGGCAAGGTGTTAAGCACTTTAGTGCGAGAC-CCTGCCA 114
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 55  ATAGGTGCGCAGACGTCTTGTAGCGGGGTGTTAAGCACTTGAGTGCAACACTCCGGCC- 113

Query 115  CAAGACGCTAGCTACAGCTCCCTAGGGTGTGATGAGTGCTTTAGCTGGCACTCATCCACT 174
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 114  TAAGACGCTAGCTATGGCTTCGTAGGGTGTGGTGTGAGTGCTTTA ACCAGCACTCATCGACC 173

Query 175  ACCCA GCCCACCTACCCTTGGGACAA GGGCTGTGC-TACCTCTGCGCAGTCCTGTCCGGT 233
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 174  GCCCT GCCTACCTACCCTTGTGATAC GAGCCTCGCAGATTCCTGCGTGGCCCGTGTCCG- 232

Query 234  TTGTATATCACTCCTGAGCTA 254
          ||| ||| ||| ||| ||| ||| |||
Sbjct 233  TTGTATATCACTCCTGAGCTA 253
          ||| ||| ||| ||| ||| ||| |||

```

***Andreaea rupestris* locus 2010369 (accession WOGB\_2010369) (E-value: 2e-42)**

```

Query 1  GGCGGTAACCCCTTCTGAGCTAAGTAAGCTGGGGGTGGGT---GGAGCCAAGTAGAGGAGG 57
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 1  GGCGGTAATCCCTGCTGAGCTAAGCAAGGGGGAGGTTGGTTCGCGGGGC--ACTAGT--AGG 56

```

```

Query 58 TTTGTAGTGCATC-TTGTAGGCAAGGTGTTAAGCACTTTAGTGCAGACCCTGCCACA 116
          ||||| | ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 57 C--GTAGTG-GATTCCTTGACGGTGGGGTGGGA-GTTCTTTAGTGCAGACCCTGTCGCA 112

Query 117 AGACGCTAGCTACAGCTCCCTAGGGGTGTGATGAGTGCTTTAGCTGGCACTCATCCACTAC 176
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 113 AGACGTCAGCTATGGCTCCC TAGGGGTGTGATGAGTGCTTTAGCCAGCACCCCTACGTTAC 172

Query 177 CCAGCCACCTACCCTTGGGACAA--GGGCTGT-GCTACCTCTGCGCAGTCCCTGTGCGGT 233
          ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 173 CCAGCCACCTACCCTTGGGACAA GAGGGGTATAGGCAACTATGCGC--CCCTTGTGTCAGG 230

Query 234 TTGTATATCACTCCTGAGCTA 254
          | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 231 TCGATTATCACTCCTGAGCTA 251

```

**Figure 4**(on next page)

Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-like loci from mosses with class II TAS3 loci 14-Aru (A) and WOGB\_2010369 (B) of *Andreaea rupestris*.

BLASTN was used at NCBI blast site. The miR390 target sites are in yellow; putative tasiARF-a2 site is in green; tasiAP2 is in blue.

## (A)

**Andreaea rupestris locus 2010369 (accession WOGB\_2010369) (E-value: 5e-16)**

```
Query 1  GCGGTAACCCCTTCTGAGCTACGT---GGGTAGCTAGCTGGTCGCGGGGCACTAGTA  54
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1  GCGGTAACCCCTTCTGAGCTAAGCAAGGGGGAGGT---TGGTCGCGGGGCACTAGTA  54

Query 90  ...CTTTAGTGTGAGACCCGTGCTTCCATTCACTAGGAATGGTGGGC TAGGGTGTGATGAGTGC  149
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 90  ...CTTTAGTGTGAGACCCGTGCGAAGACGTCAGCTATGGCTCCC TAGGGTGTGATGAGTGC  149

Query 150  TTTAGCCAGCACTTCATCACA AATTTGCTGCCTACCCTTGGGACATCTTCTGTCTACCC  209
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 150  TTTAGCCAGCACCCCTTACGTTACCCA GCCACCTACCCTTGGGACAA G-----  187

Query 210  TCGGACAGGGGGGCGATAGCTTGCTATGCTGCCCTTGTCAGTTGTCTATCACTCCTGA  268
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 188  -----AGGGGTATAGGCAACTATGCGCCCTTGTCAGGTCGATTATCACTCCTGA  247

Query 269  GCTA  272
         |||
Sbjct 248  GCTA  251
```

**Tetraphis pellucida clone 73-Tpe (accession KC812754) (E-value: 2e-28)**

```
Query 1  GCGGTAACCCCTTCTGAGCTACGTGGGTAGCTAGCT-GGTCGCGGGGCACTAGTACAAGC  59
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1  GCGGTAACCCCTTCTGAGCTAAGTGGGTAGGGGGCTTGGTCGCGGGGCACTAGTACACGC  60

Query 60  TACCACCTCCTAAGCGAG-ACGATACTTGAC- TTTAGTGTGAGACCCGTGCTTCCATTCA  117
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 61  GGGAACACCCTGAAAAATGTAGGAGTGTTCGCCGTTTAGTGCAAGGCCCACTTCCAATAG  120

Query 118  CTAGGAATG-GTGGGCTAGGGTGTGATGAGTGCCTTA GCCAGCACTTCATCACA AATTTG  176
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 121  TTAGGATGAGCCATT TAGGGTGTGATGAGTGCCTTA GGCAGCACTTTCTCAA ACCCAA  180

Query 177  CTGCCTACCCTTGGGACATCTTCTGTCTACCCTCGGACAGGG--GGGGCA-TAGCTTGC  233
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 181  CCAG-CTACCCTTGAGACAAGGCCCGTCTACCCTTGGGCAAGGTCTTTGCATTACCTGC  239

Query 234  TATGCTGCCCTTGTCAGTTGTCTATCACTCCTGAGCTA  272
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 240  T---CGGCCCTTGTGTTTGTCTATCACTCCTGAGCTA  275
```

**Physcomitrella patens cluster TAS3b (accession BK005826) (E-value: 2e-08)**

```
Query 1  GCGGTAACCCCTTCTGAGCTA  21
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 5  GCGGTAACCCCTTCTGAGCTA  25

Query 130  GGCTAGGGTGTGATGAGTGCCTTA GCCAGCACTTCATCACA AATTTGCTGCCTACCCTT  189
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 56  GGCTAGGGTGTGATGAGTGCCTCC GTAAGCAACTCATGCGAGCTCA ACCA-CGTACCCTT  114

Query 190  GGGACATCTTCTGTCTACCCTCGGACAGGGGGGCGATAGC-TTGTATGCTGCCCTTGTG  248
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 115  GAGACAA GACCCGTCTGCCATCGCACAAGGGCAACGCACCAATTGCG-TGA-GCCCTTGT  172

Query 249  AGTTGTCTATCACTCCTGAGCTA  272
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 173  GTTTGTCTATCACTCTGAGCTA  196
```

## (B)

**Tetraphis pellucida clone 80-Tpe (accession KC812753) (E-value: 2e-20)**

```
Query 1  GCGGTAACCCCTTCTGAGCTAAG----CAAGGGGGAGGTTGGTCGCGGGGCACTAGTAGG  56
         ||| | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1  GCGGTAACCCCTTCTGAGCTAAGTAGCCAAGGTTAGCT--GTAG---GGCAGTAGGAGC  55
```

```

Query 57  CGTAGT--GGATTCTTGACGGTGGGGTGGGA-GTTCTTTAGTGCGAGACCCTGTGCGCAA 113
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 56  CTTTGCCGGGGGTC-TTGTAGCGGGGTGTTAAGCACTCGCGTGC GGCTCTCCGTGGTAA 114

Query 114 GACGTCAGCTATGGCTCCC TAGGGTGTGATGAGTGCTTTAGCCAGCACCCCTTAC-GTTAC 172
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 115 GACGTCAGCTATGGCTCCG TAGGGTGTGATGAGTGCTTTACCTGGCGCTC-AACAACCTTC 173

Query 173 CCAGGCCACCTACCCCTGGGACAAAG---AGGGGTATAGGCAACTATGCGCCCTTGTC 228
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 174 CCAGGCCACCTACCCCTGGGACACGGGCCAAGTGGAT-TTCCACT---CGGCCTGTGTC- 228

Query 229 GGTTCGATTATCACTCCTGAGCTA 251
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 229 GGTTCGATTATCACTCCTGAGCTA 251

```

***Bartramia halleriana* clone 29-Bha (accession KC812746) (E-value: 1e-23)**

```

Query 1  GGCGGTATCCCTGCTGAGCTAAGCAAGGGGG--AGGTTGGTCGCGGGGCACTAGTA-GGC 57
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 1  GGCGCTATCCCTCCTGAGCTAGAAAAGAAAGGCAAGGGGCCCTCCGGGGGCGATTATGGT 60

Query 58  GTAGTGGATTCCCTTGACGGTGGGGTGGGA-GTTCTTTAGTGCGAGACCCTGTGCGAAGAC 116
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 61  GAAGCGGATGCCTTGTTAGCGGGGTGTTAAGCACTTGAGTACGACACTCGGGCCCTTGAC 120

Query 117 GTCAGCTATGGCTCCC TAGGGTGTGATGAGTGCTTTAGCCAGCACCCCTTACGTTACCCAG 176
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 121 CTCCGCTATGGCTTCG TAGGGTGTGATGAGTGCTTTACCCGGCGCTCATCCACTGCCAG 180

Query 177 CCCACCTACCCCTGGGACAAAGAGGGGTATAGGCA-A-CTATGCGC--CCCTTGTCAGGTC 232
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 181 CCCACCTACCCCTGTGACAT---GGGCA-CCGAGATCCCTGCGCTGCCCTTGTC-GGTT 235

Query 233 GATTATCACTCCTGAGCTA 251
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 236 GTATATCACTCCTGAGCTA 254

```

***Tetraphis pellucida* clone 73-Tpe (accession KC812754) (E-value: 1e-13)**

```

Query 1  GGCGGTATCCCTGCTGAGCTAAGCAAGGGGGAGG-TTGGTCGCGGGGCACTAGTAGGCGT 59
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 1  GGCGGTAAACCCTTCTGAGCTAAGTGGGTAGGGGGCTTGGTCGCGGGGCACTAGTACACG- 59

Query 60  AGTGGATTCCCTTGACGGTGGGGTGGGAGTTC---TTTAGTGCGA-GACCCTGTGCGAAG 114
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 60  CGGGAACACCCTGAAAATGTAG-GAGTGTTCGGGTTAGTGCAAGGCCCACTTCCAAT 118

Query 115 ACGTCAGCTATG-GCTCCC TAGGGTGTGATGAGTGCTTTAGCCAGCACCCCTTACGTTACC 173
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 119 A-GTTAGGGATGAGCCATT TAGGGTGTGATGAGTGCTTTAGCCAGCA-CTTTCTCAAACC 176

Query 174 CAGCCACCTACCCCTGGGACAAAG 197 ... 236 TATCACTCCTGAGCTA 251
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 177 CCAACCAGCTACCCCTGGGACAAAG 200 ... 260 TATCACTCCTGAGCTA 275

```

***Timmia austriaca* clone 2061439 (accession ZQRI\_2061439) (E-value: 3e-29)**

```

Query 1  GGCGGTATCCCTGCTGAGCTAAGCAAGGGGG--AGGTTGGTCGCG--GGGCACTAGTA-G 55
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 1  GGCGCTATCCCTCCTGAGCTAGAAAAGAAAGGCAAG--GGTCCCTCCGGGGGCGATTATG 58

Query 56  GCGTAGTGGATTCCCTTGACGGTGGGGTGGGA-GTTCTTTAGTGCGAGACCCTGTGCGAAG 114
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 59  GTGAAGCGGATGCCTTGTTAGCGGGGTGTTAAGCACTTGAGTTCGACACTCGGGCCCTTG 118

Query 115 ACGTCAGCTATGGCTCCC TAGGGTGTGATGAGTGCTTTAGCCAGCACCCCTTACGTTACCC 174
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||
Sbjct 119 ACCTCCGCTATGGCTTCG TAGGGTGTGATGAGTGCTTTACCCGGCGCTCATCCACTGCC 178

Query 175 AAGCCACCTACCCCTGGGACAAAGAGGGGTATAGGCA-A-CTATGCGC--CCCTTGTCAGG 230
          ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| ||| |||

```

```
Sbjct 179 AGCCACCTACCCTTGTGACA---GGGCA-CCGCAGATCCCTGCGCTGCCCTTGTC-GG 233
Query 231 TCGATTATCACTCCTGAGCTA 251
          | | ||||| ||||| |||||
Sbjct 234 TTGTATATCACTCCTGAGCTA 254
```

**Figure 5**(on next page)

Multiple sequence alignments of nucleotide sequence blocks including tasiAP2 site and preceding 21 bp site of putative ta-siRNA of *Andreaea rupestris* TAS3-like locus WOGB\_2010369.

BLASTN was used at 1KP blast site. For the complete TAS3 transcript sequences see Xia et al., 2017. The putative tasiAP2 site is in blue, and preceding putative ta-siRNA site is in violet.

*Andreaea1* - *Andreaea rupestris* WOGB\_2010369; *Andreaea2* - *Andreaea rupestris* WOGB\_2002765; *Tetraphis1* - *Tetraphis pellucida* HVBQ\_2019753; *Tetraphis2* - *Tetraphis pellucida* HVBQ\_2011866; *Tetraphis3* - *Tetraphis pellucida* HVBQ\_2005644; *Plagiomnium* - *Plagiomnium insigne* BGXB\_2010105; *Leucobryum* - *Leucobryum glaucum* RGKI\_2062694; *Racomitrium* - *Racomitrium varium* RDOO\_2117129; *Philonotis* - *Philonotis fontana* ORKS\_2058791; *Dicranum* - *Dicranum scoparium* NGTD\_2078536; *Encalypta* - *Encalypta streptocarpa* KEFD\_2058811; *Ceratodon* - *Ceratodon purpureus* FFPD\_2044193; *Niphotrichum* - *Niphotrichum elongatum* ABCD\_2000143; *Funaria* - *Funaria* sp. XWHK\_2042016; *Schwetschkeop* - *Schwetschkeopsis fabronia* IGUH\_2166854; *Aulacomnium* - *Aulacomnium heterostichum* WNGH\_2088134; *Syntrichia* - *Syntrichia princeps* GRKU\_2074985; *Diphyscium1* - *Diphyscium foliosum* AWOI\_2069791; *Diphyscium2* - *Diphyscium foliosum* AWOI\_2006305; *Hypnum* - *Hypnum subimponens* LNSF\_2068452; *Pohlia* - *Pohlia nutans* GACA01023180; *Bryum* - *Bryum argenteum* GCZP01053768.



<b>Andreaea1</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	43
<b>Andreaea2</b>	AAGACGCTAGCTACAGCTCCCTAGGGTGTGATGAGTGCTTTA	190
<b>Tetraphis1</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	137
<b>Tetraphis2</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	182
<b>Tetraphis3</b>	AAGACGCCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	440
<b>Plagiomnium</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	315
<b>Leucobryum</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	1006
<b>Racomitrium</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	304
<b>Philonotis</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	314
<b>Dicranum</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	58
<b>Encalypta</b>	AAGACGTCAGCTATGGCTCCATAGGGTGTGATGAGTGCTTTA	1009
<b>Ceratodon</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	192
<b>Niphotrichum</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	455
<b>Funaria</b>	AAGACGTCAGCTATGGCTCTGTAGGGTGTGATGAGTGCTTTA	250
<b>Schwetschkeop</b>	AAGACGTCAGCTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	1632
<b>Aulacomnium</b>	AAGACGTCAGTTATGGCTCCCTAGGGTGTGATGAGTGCTTTA	278
<b>Syntrichia</b>	AGGACGTCAGCTATGGTTCCCTAGGGTGTGATGAGTGCTTTA	154
<b>Diphyscium1</b>	AAGACGTCAGCTATGTCTTCATAGGGTGTGATGAGTGCTTTA	1047
<b>Diphyscium2</b>	AAGACGTCACCTTATGGCTTCCTAGGGTGTGATGAGTGCTTAA	486
<b>Hypnum</b>	AGACGTCACCCATGGCTCCCTAGGGTGTGATGAGTGCTTTAA	1485
<b>Pohlia</b>	AAGACGCCAGCTATGGCTTCCTAGGGTGTGATGAGTGCTTTA	38
<b>Bryum</b>	AAGACGCCAGCTACGGCTTCCTAGGGTGTGATGAGTGCTTTA	240

**Figure 6**(on next page)

Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-like loci from mosses with TAS3 of *Takakia lepidozoides*.

BLASTN was used at NCBI blast site. The miR390 target sites are in yellow; putative tasiARF-a2 site is in green; tasiAP2 is in blue.

***Andreaea rupestris* locus 13-Aru (accession KC812744) (E-value: 2e-23)**

```

Query  1  GGCGCTAACCTTCCTGAGCTAAGCCAGTAGAGGGTGGGTTGAG  43
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  1  GGCGGTAACCCCTTCTGAGCTAAGTAAGCTGGGGTGGGTGGAG  43

Query  88  CCTAGGGTGTGATGAGTGCTTTA--CCAGCACCTCA--CATTGGCCCA  144
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  135  CCTAGGGTGTGATGAGTGCTTTAGCTGGCA-CTCATCCACT-ACCCA  192
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

Query  145  TGGTACAAAGGGGACTGCAACTTTTTCGCCATCCTTGTAATAA  204
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  193  TGGGACAAAGGGGACTGCAACTTTTTCGCCATCCTTGTAATAA  251
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||

Query  205  CTA  207
          |||
Sbjct  252  CTA  254
          |||

```

***Tetraphis pellucida* clone 80-Tpe (accession KC812753) (E-value: 2e-14)**

```

Query  1  GGCGCTAACCTTCCTGAGCTAAG  23
          ||||| ||||| ||||| ||||| |||||
Sbjct  1  GGCGGTAACCCCTTCTGAGCTAAG  23

Query  90  TAGGGTGTGATGAGTGCTTTA--CCAGCACCTC--ACATTGGCCCA  148
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  134  TAGGGTGTGATGAGTGCTTTACCTGGCGCTCAACAACCTCCCA  193
          ||||| ||||| ||||| ||||| ||||| ||||| |||||

Query  149  ACAAGGGGACTGCAACT--TTTTGCGC-CATCCT-TGTAATAA  204
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  194  ACACGGG--C--CAAGTGGATTTCACCTCGGCTGTGT-CGG  248
          ||||| ||||| ||||| ||||| ||||| ||||| |||||

Query  205  CTA  207
          |||
Sbjct  249  CTA  251
          |||

```

***Timmia austriaca* clone 9-Tau (accession KC812755) (E-value: 6e-22)**

```

Query  1  GGCGCTAACCTTCCTGAGCT  20
          ||||| ||||| ||||| ||||| |||||
Sbjct  1  GACGCTAACCCCTTCCTGAGCT  20

Query  90  TAGGGTGTGATGAGTGCTTTA--CCAGCACCTCA--CATTGGCCCA  147
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  137  TAGGGTGTGATGAGTGCTTTACCCGGCGCTCATCCACT-GCCCA  195
          ||||| ||||| ||||| ||||| ||||| ||||| |||||

Query  148  TACAAAGGGGACTGCAACTTTTTCGCCATCCTTGTAATAA  207
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  196  GACATGGGCACCGCAGATCCCTGCGCTGTCTTGT-CGG  254
          ||||| ||||| ||||| ||||| ||||| ||||| |||||

```

***Polytrichum commune* clone 122-Pco (accession KC812751) (E-value: 2e-13)**

```

Query  1  GGCGCTAACCTTCCTGAGCTA  21
          ||||| ||||| ||||| ||||| |||||
Sbjct  1  GGCGGTAACCCCTTCTGAGCTA  21

Query  89  CTAGGGTGTGATGAGTGCTTTA--CCAG-CACCTCA-CATTGGCCCA  146
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  109  CTAGGGTGTGATGAGTGCTTAAAGCTGGCATCTCCTCAATG-CCCA  166
          ||||| ||||| ||||| ||||| ||||| ||||| |||||

Query  147  GTACAAAGGGGACTGCAACTTTTTCGCCATCCTTGTAATAA  207
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct  167  AGACAAAGAGCTTTGCAGTTACATGCAAGGCCCTTGTGTT  227
          ||||| ||||| ||||| ||||| ||||| ||||| |||||

```

***Bryum pseudotriquetrum* clone 72-Pps (accession KC812758) (E-value: 3e-18)**

```

Query  1  GGCGCTAACCTTCCTGAGCTAAG  23
          ||||| ||||| ||||| ||||| |||||
Sbjct  1  GGCGTTATCCCTCTTGAGCTGAG  23

Query  90  TAGGGTGTGATGAGTGCTTTA--CCAGCACCTCA-CATTGGCCCA  148
          ||||| ||||| ||||| ||||| ||||| ||||| |||||

```

```
Sbjct 129 TAGGGTGTGATGAGTGCTTTACCAGGCGCTCATCCTCTACCCAGCCCACCTACCCCTGTG 188
Query 149 ACAAGGGGACTGCAACTTT--TTGCGCCATCCTTGTAATTTGTTTATCACTCCTGAGCTA 207
Sbjct 189 ACATGGG--CCGCTCCCTTCCCGGCGGGCCCTTGTCAA-TTGTCATCACTCCTGAGCTA 246
```

**Figure 7** (on next page)

Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-like loci from mosses with TAS3 of hornwort *Folioceros fuciformis*.

BLASTN was used at NCBI blast site. The miR390 target sites are in yellow; putative tasiARF-a2 site is in green; tasiAP2 is in blue; tasiARF-a3 is shaded.

***Physcomitrella patens* cluster TAS3a (accession BK005825) (E-value: 4e-26)**

```
Query 1 GCGGTTATCCTTCCTGAGCTGAGAA-AGAAGG-----CAAGGGTGGGGG--TGCCGT 49
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1 GCGGTTATCCTTCCTGAGCTGAGAAGACAAGGGCTCCTCCTAGGGGGCGAAAATAG-GT 59

Query 50 G-GCGGGCGGGCGCCTTGTAAACGGGGGTGTTAAGCACCA-----ACGGACGCCCTGGCA 101
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 60 GAGCTGGGGTACACCTGTTAGCGGGGTGTTAAGCATTGAATGCAAC--ACTCCTACGCA 117

Query 102 GCCTCAGACGCCACCCACGGCTCCGTAGGGTGTGATGAGTGCTTTACCTAGCGCTAGCC 161
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 118 -----AGACCCTAGCTATGGCTCCA TAGGGTGTGATGAGTGCTTCATCCGGTGTCTTCT 172

Query 162 CCTGGCGAGCCACCTACCCCTGTGACACGGGCCTGGCAGATCCCTGCACGGCCCCTG-- 219
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 173 ACTGCCTTGCACCTACCCCTGTGATAIGGGCCGCGC-G-TGTCTGCGTGTCTCCTGTA 230

Query 220 TCGGTTACGTATCACTCCTGAGCTA 244
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 231 TCGGTTGTATATCACTCCTGAGCTA 255
```

***Physcomitrella patens* cluster TAS3d (accession BK005828) (E-value: 6e-20)**

```
Query 1 GCGGTTATCCTTCCTGAGCTGAGAAAGAAGGCAAGGG-----TGGGGGTG--GCGTGGC 52
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1 GCGGCTATCCCTCCTGAGCTTAGAAAGAAGTCAAGGGCTCCTTGGGAGCAAAGCCAGGT 60

Query 53 GGG----CGGCGCCTTGTAAACGGGGGTGTTAAGCACCAACG---GACGCCCTGGCAGCCT 105
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 61 GGGTCGACGTGCGCTTGTAAAGCGGGGTGTTATGCACTTGCCTTGACGCTCCG--ATC-T 117

Query 106 CAGACGCCACCCACGGCTCCGTAGGGTGTGATGAGTGCTTTACCTAGCGCTCAGCCCCTG 165
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 118 AAGACGTACAGTGTAGCTCCATGCGGGTGTGACTGCTTAGCCCGGCACACACTCTCTG 177

Query 166 GCGAGCCACCTACCCCTGTGACACGGGCCTGGCAGATCCCTGCACGGC--CCCTGTCCG 223
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 178 CCCGCGCCACCTACCCCTGTGAGATGGGTAGCGCTGAT--TTGCGCGACTTCCATGTCCG 235

Query 224 TTACGTATCACTCCTGAGCTA 244
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 236 TTTTATATCACTCCTGAGCTA 256
```

***Encalypta rhoptocarpa* clone 35-Erh (accession KC791767) (E-value: 2e-29)**

```
Query 1 GCGGTTATCCTTCCTGAGCTGAGAAAGAAGGCAAGGGT-----GGGGGTGGCGT-GGCGG-G 55
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1 GACGCTACCCTTCCTGAGCTGAGAAAGAAGCTAGGGGTCCTTGAGAGGGGAATAGGTGGAG 62

Query 56 -CGGCGCCTTGTAAACGGGGGTGTTAAGCACCAACGGAC-GCC-CTGGCAGCCTCAGACGC 112
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 63 TCGACGCCTTGTAAAGCGGGGTGTTACGCACAAG-GGTGTGCCACTCCC-GCCTAAGACGT 120

Query 113 CACCCACGGCTCCGTAGGGTGTGATGAGTGCTTTACCTAGCGCTCAGCCCCTGGCGAGCC 172
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 121 CAGCTATGGCTCTGTAGGGTGTGATGAGTGCTTTACCGAGCGCTCATCCACCGCCTTGAA 180

Query 173 CACCTACCCCTTG-TGACACGGGCCTGGCAGATCCCTGCACGGCCCCT-GTCGGTTACGTA 230
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 181 CACCTACCTTAGCAGACACGGCCCTCGCAGTTCTTGCCTGGCCCGTGGTC-ATTGTCCTA 239

Query 231 TCACTCCTGAGCTA 244
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 240 TCACTCCTGAGCTA 253
```

***Bryum argenteum* clone 9-Bar (accession KC812760) (E-value: 3e-33)**

```
Query 1 GCGGTTATCCTTCCTGAGCTGAGAAAGAAGGCAAGGGTGGGGGTGGCGTGGCCGGGCGGCG 60
| | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
Sbjct 1 GCGGTTATCCCTTCCTGAGCTGAGAACGACGACGAGGGAGGG-----CTCGGCCG----- 49
```

```

Query 61 CCTTGTTAACGGGGTGTTAAGCACCAACGGACGC-CCTGGCAGCCTCA-GACGCCACCCA 118
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 50 CCTTGTTAGCGGGTGTTAAGCACTTCTGTGCGAACCTC-CATC-TCAAGACGCCAGCTA 107

Query 119 CGGCTCCGTAGGGTGTGATGAGTGCTTTACCTAGCGCTCAGCCCCTGGCGAGCCCACCTA 178
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 108 CGGCTTCGTAGGGTGTGATGAGTGCTTTAATGGCGCTCATCTCTACCCAAGCCCACCTA 167

Query 179 CCCTTGTGACAGGGCCTGGCAGATCCCTGCACGGCCCTGTCCGTTACGTATCACTCCT 238
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 168 CCCTTGTGACATGGCCGCTCCCTTCCGGTTCGGCCCTTGTCAAATTGTCTATCACTCCT 227

Query 239 GAGCTA 244
          |||||
Sbjct 228 GAGCTA 233
          |||||

```

***Timmia austriaca* clone 9-Tau (accession KC812755) (E-value: 2e-44)**

```

Query 3 CGTTATCCTTCCTGAGCTGAGAAAGAAGGCAAGGG-----TGGGGTGGCG----TGGCG 53
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 3 CGTACCCTTCCTGAGCTGAGAAAGAAGGCAAGGGGCCCTCCGGG-GGCGATTATGGTG 61

Query 54 G-GCGGC-GCCTTGTTAACGGGGTGTTAAGCACCAACGGACGCC-CTGGCAGCCTCAGAC 110
          | ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 62 GAGCGGATGCCTTGTAGCGGGTGTTAAGCACTTGAGTACGACACTCGGGCCCTT-GAC 120

Query 111 GCCACCCACGGCTCCGTAGGGTGTGATGAGTGCTTTACCTAGCGCTCAGCCCCTGGCGAG 170
          | ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 121 CTCCGCTATGGCTTCGTAGGGTGTGATGAGTGCTTTACCCGGCGCTCATCCACTGCCAG 180

Query 171 CCCACCTACCCTTGTGACAGGGCCTGGCAGATCCCTGCACGGCCCTGTCCGTTACGTA 230
          ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| ||||| |||||
Sbjct 181 CCCACCTACCCTTGTGACATGGGCACCGCAGATCCCTGCGCTGCCTTGTCCGTTGTATA 240

Query 231 TCACTCCTGAGCTA 244
          |||||
Sbjct 241 TCACTCCTGAGCTA 254
          |||||

```



**Figure 8**(on next page)

Pairwise sequence comparisons of selected available nucleotide sequences of TAS3-like loci from non-vascular plants with TAS3 of *Marchantia polymorpha*.

BLASTN was used at NCBI blast site. The miR390 target sites are in yellow; putative tasiARF-a1 site is in brown; tasiAP2 is in blue.

***Tetraphis pellucida* clone 80-Tpe (accession KC812753) (class Tetraphidopsida)**

Query 1 GGCGGTATCC-TTCTTGAGCTAA 22  
 |||||  
 Sbjct 1 GGCGGTAACCTTCT-GAGCTAA 22

Query 161 AGGGTGTGATGAGTGCTTTACCTGG 185  
 |||||  
 Sbjct 135 AGGGTGTGATGAGTGCTTTACCTGG 159

Query 237 TGCCTATCACTCTTGAGCTA 256  
 |||||  
 Sbjct 232 TGTCTATCACTCCTGAGCTA 251

***Takakia lepidozoides* (accession SKQD-2076588) (class Takakiopsida)**

Query 1 GGCGGTATCCTTCTTGAGCTAA 22  
 |||||  
 Sbjct 1 GGCGCTAACCTTCCTGAGCTAA 22

Query 161 AGGGTGTGATGAGTGCTTTACC 182  
 |||||  
 Sbjct 91 AGGGTGTGATGAGTGCTTTACC 112

Query 241 TATCACTCTTGAGCTA 256  
 |||||  
 Sbjct 192 TATCACTCCTGAGCTA 207

***Folioceros fuciformis* (accession SRX2779513) (class Anthocerotopsida)**

Query 1 GGCGGTATCCTTCTTGAGCTA-AAAAGA 27  
 |||||  
 Sbjct 1 GGCGTTATCCTTCCTGAGCTGAGAAAAGA 28

Query 161 AGGGTGTGATGAGTGCTTTACCT 183  
 |||||  
 Sbjct 128 AGGGTGTGATGAGTGCTTTACCT 150

Query 241 TATCACTCTTGAGCTA 256  
 |||||  
 Sbjct 229 TATCACTCCTGAGCTA 244

***Marchantia emarginata* (accession SRX1952816) (class Marchantiopsida) (E-value: 6e-60)**

Query 3 CCGTATCCTTCTTGAGCTA---A-AA--AGATGTAGCTTCCTGCTACATCTCACACGACA 56  
 |||||  
 Sbjct 3 CCGTATCCTTCTTGAGCTAGGAAGAAGGAGATGTAGCTTCCTGCTACATCTCACACGACA 62

Query 57 ATCTCATTTGAATGTTCAAACCTTTAGTGACTG-AA-TCGAATACTAAAGTTAATTTGA 114  
 |||||  
 Sbjct 63 GTCTCGTTTGATGTTCAAATCTCTCG-GA-TGTAAGTCACATACAGAAGTTAATTTGA 120

Query 115 CTTCAATAGAGACTAGTTTGC GGAGAAACTGTGCCAGTTAGCAGGAGGGTGTGATGAGT 174  
 |||||  
 Sbjct 121 CGGCAAGCGAGACACATGTGCGGGACGGACACCCTGGTTAGCATGAGGGTGTGATCAGT 180

Query 175 GCTTTACCTGGTCCAGGATCCCCACCCCTCCTCCACTGCCTATTTCTAGGCTCGCGTTA 234  
 |||||  
 Sbjct 181 GCTTTACCCGGTCCGGTTC CCGTCCCCTTCCCCACTGCCTATGTCTAGGCTCGCCTGA 240

Query 235 CCTGCCTATCACTCTTGAGCTA 256  
 |||||  
 Sbjct 241 CCTGCCTATCCCTCTTGAGCTA 262

***Conocephalum japonicum* (accession SRX1952810) (class Marchantiopsida) (E-value: 1e-25)**

Query 3 CCGTATCCTTCTTGAGCTA-----AAA-AGATGTAGC-----TT-CCTGCTACAT 45  
 |||||  
 Sbjct 3 CCGTATCCTTCTTGAGCTAGGAGGAAAGAGATGTAGCGAGCGGGACCTTACCCTGCTACAT 62

Query 46 CTCACACGACAATCTCATTTGAATGTTCAAACCTTTAGTGACTGAATCGAATACTAAAG 105

```

Sbjct 63  CTCACACGACG|GTCTCGTTTGAATGTGCA-----TG--TC---AC--AAT 101
Query 106  TTAATTTGACT-TCAATAGAGACTAGTTTGC|GGGAGAACTGTGCCAGTTAGCAGGAGGG 164
          |||||  ||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 102  TTAATTTGGATGATCAAC-GAGATAAATGTGTTGGATGGACTCTCCTGGCTAGCATGAGGG 160
Query 165  TGTGATGAGTGC|TTTACCCTGGTCCAGGA-TCC|CCACCCCTCCTCCACT-GCCTATTTCT 222
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct 161  TGTGATGAGTGC|TTTACCA--TACAGGGGTTCCCGTCTTTCTTCCCGTCGCATATGTCT 218
Query 223  AGGCTCGCGTTAC|CTGCCTATCACTCTTGAGCTA 256
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 219  AGGTTTCGCTGAC|CTGCCTATCCCTCTTGAGCTA 252
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||

```

***Ricciocarpus natans* (accession ERX337127) (class Marchantiopsida) (E-value: 3e-20)**

```

Query 1  GGCGGTATCCTTCTTGAGCTA|AAAAAGAT-GTAGCTTC|CTGCTACATCTCACACGACATCT 61
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct 1  GACGGTATCCTTCTTGAGCTA|AGGGGACAGGAAAT-CTGCTACATCTCACACGATAGTTT 61
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Query 62  CATTTGAATGTTCAAACCTTTAGTGACTGAATCGAA--TACTAAAGTTAA-TTTGACTTC 118
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 62  CTTTGGTATCTTCAAATC---AGAATATGATTGGAAGATACACGAGATAAATTG----- 113
Query 119  AATAGAGACTAGTTTGC|GGGAGAACTGTGCCAGTTAGCAGGAGGGTGTGATGAGTGC|TT 178
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 114  --T-GATAC-----GGACAC-CTCTGCC---TAAATGAGGGTGTGATGAGTGC|TT 157
Query 179  TACCTGGTCCAGGATCCCCACCCCTCCT-CCACTGCCTATTTCTAGGCTCGCGTTACCT 237
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 158  TA-CTAGGCAGGGGTTACGTC|CATTTCTCCACTGCATATGTCTAGGTTCGCCTGACCT 216
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Query 238  GCCTATCACTCTTGAGCTA 256
          ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 217  GCCTATCCCTCTTGAGCTA 235
          ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||

```

***Dumortiera hirsuta* (accession SRX1126014) (class Marchantiopsida) (E-value: 2e-39)**

```

Query 3  CGGTATCCTTCTTGAGCTA--A--AA--AGATGTAGCT--TC|CTGCTACATCTCACACGA 54
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct 3  CGGTATCCTTCTTGAGCTA|GGAGGAACGAGATGTAGCTGTTCT|CTGCTACATCTCACACGA 62
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Query 55  CACATCTCATTTGAATGTTCAAACCTTTAGTGACTGAATCGAATACTAAAGTTAATTTGA 114
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct 63  CATGTCTCATTGTATGTTCAA-----TC-AA----AAA-TTAATTTGA 101
Query 115  CTTCAATAGAGACTAGTT-TGCGGGA--GAAAC--TGTGCCAGTTAGCAGGAGGGTGTGA 169
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 102  CAACACACGAGAC-AGACGTGAGGGACAGAGACCTTTTGC---TAGCATGAGGGTGTGA 156
Query 170  TGAGTGC|TTTACCCTGGTCCAGGATCCCCACCCCTCCT-CCACTGCCTATTTCTAGGCTC 228
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct 157  TGAGTGC|TTTACCAGG-CAAGGGTTCAGTCTTTTCTCCATTGCCTATGCTTAGGCTC 215
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Query 229  GCGTTACCTGCCTATCACTCTTGAGCTA 256
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 216  GCCTGACCTGCCTATCCCTCTTGAGCTA 243
          |||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||

```

***Plagiochasma appendiculatum* (accession SRX1741567) (class Marchantiopsida) (E-value: 5e-34)**

```

Query 3  CGGTATCCTTCTTGAGCTA-----AAA-AGATGTAGC---T--TC|CT-GCTACATCTCAC 50
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct 3  CGGTATCCTTCTTGAGCTA|GGAGGAAAAGAGATGTAGCGTTTAGTG|CTAGCTACATCTCAC 62
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Query 51  ACGACATATCTCATTTGAATGTTCAAACCTTTAGTGACTGAATCGAATACTAAAGTTAAT 110
          |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||  |||||
Sbjct 63  ACGACATGTCTCGTTTGTGTTTCAAATC---AG-----AAGTCAAT 101
Query 111  TTGACTCAATAGAGACTAGTTTGC|GGGAGAACTGT-GCCAGTT---AGCAGGAGGGTGTG 166
          ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||
Sbjct 102  TTGATGACAAAAGAGAC-AGA---CGTGTGAGACGGACGCCTTTGGCCAGCATGAGGGTGTG 157
          ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||  ||||

```



**Figure 9** (on next page)

Pairwise sequence comparisons of selected nucleotide sequences of TAS6/TAS3-like loci from mosses with TAS6/TAS3 of *Physcomitrella patens* precursor RNA (accession JN674513).

BLASTN was used at 1KP blast site. The miR390 target sites are in yellow; putative miR156/miR529 sites are underlined; tasiAP2 is in blue; putative tasiARF-a2 site is in green; tasiARF-a3 is shaded.



```

Query 165 ...GTGATCACTCTCTCTGTCAA 185
          |||
Sbjct 865 ...GTGATCACTCTCTCTGTCAA 885

Query 613 TGGGCGTTATCCCTCTTGAGCTGAGAAGACAAGGGCTCCCTCCTAGG-GGGCGAAAATAG 671
          |||
Sbjct 183 TGGGCGTTATCCCTCTTGAGCTGAGAAAAGAAAGTAAGGGTG--AGGAGGGTGGAAC-G 431

Query 672 GTGAGCTGGGGTCACCTTGTAGCGGGGTGTTAAGCATTGAATGCAACACTCCTACGCA 731
          |||
Sbjct 430 G---GCCGGCGTC---TTGTAGCGGGGTGTTAAGCACTAGCGTGCACACTGCCCTTA 431

Query 732 AGACCCTAGCTATGGCTCCATAGGGTGTGATGAGTGCTTCA TCCGGTGCTCTTCTACTGC 791
          |||
Sbjct 430 AGACGTTAGCTACAACCTTGTAGGGTGTGATGAGTGCTTACCTGGTGCTATCCTCTGC 431

Query 792 CTTGCCACCTACCCTTGTGATATGGGCCGCGCGTGTCTGCG-TGTCTCCTGTATCGGT 850
          |||
Sbjct 430 CCAAGCCACCTACCCTTGTGATATGGGCCG-GCCTCTTCCCGTGCGGCCCTGTCAACT 431

Query 851 GTATATCACTCCTGAGCTA 869
          |||
Sbjct 430 GTATATCACTCCTGAGCTA 431

```

### 2069791 *Diphyscium foliosum* (accession AWOI\_2069791)

```

Query 1 ACTTTCATATGTCCTCTCTCTTCACTGTCAAGACCTCGCTT 44
          |||
Sbjct 302 ACTTTCAGATGTTCTCTCTCTCACTGTCAAGACCTCGCTT 345

Query 568 CGATGTTACGGTGTAGCCAATTCTTGTGCACTTAGATTCCACTGGCGTTATCCCTC 627
          |||
Sbjct 853 CGATGGT--GGATGTAGTCACTT-TTCTTGTA--TAGAGTACCTTCAAGCGGTATCCCTC 907

Query 628 TTGAGCTGAGAA-GA---CAAGGGCTCCCTCCTAGGGGGCGAAAATAGGTGA-GCTGGG 682
          |||
Sbjct 908 CTGAGCTGAGAAAGAGGCCAAGG---CCCT--TAGGG--CAGAAATAGGTGAAGCTGACG 960

Query 683 TCACCTGTAGT-CGGGGTGTAAAGCATTGAATGCAACACTCCTACGC-AAGACCCTAG 740
          |||
Sbjct 961 TG---TTGT-AGACTGTGTTAGACACATGAGTGTAAACACATCGG-GCTAAGACGTCAG 1015

Query 741 CTATGGCTCCA TAGGGTGTGATGAGTGCTTCA TCCGGTGCTCTTCTACTGCCTT GCCCAC 800
          |||
Sbjct 1016 CTATGCTTCA TAGGGTGTGATGAGTGCTTCA CCCGACGCTCATCTACTGCCCA GCCCAC 1075

Query 801 CTACCCTTGTGATA TGGGCCGCGCGTGTCTGCGTG-TCTCCTGTATCGGT TGTATATCAC 859
          |||
Sbjct 1076 CTACCCTTGGGACA AGGGCTGTGCAAAATTTTGTGCGGTCTT-TATCGGTGTATATCAC 1134

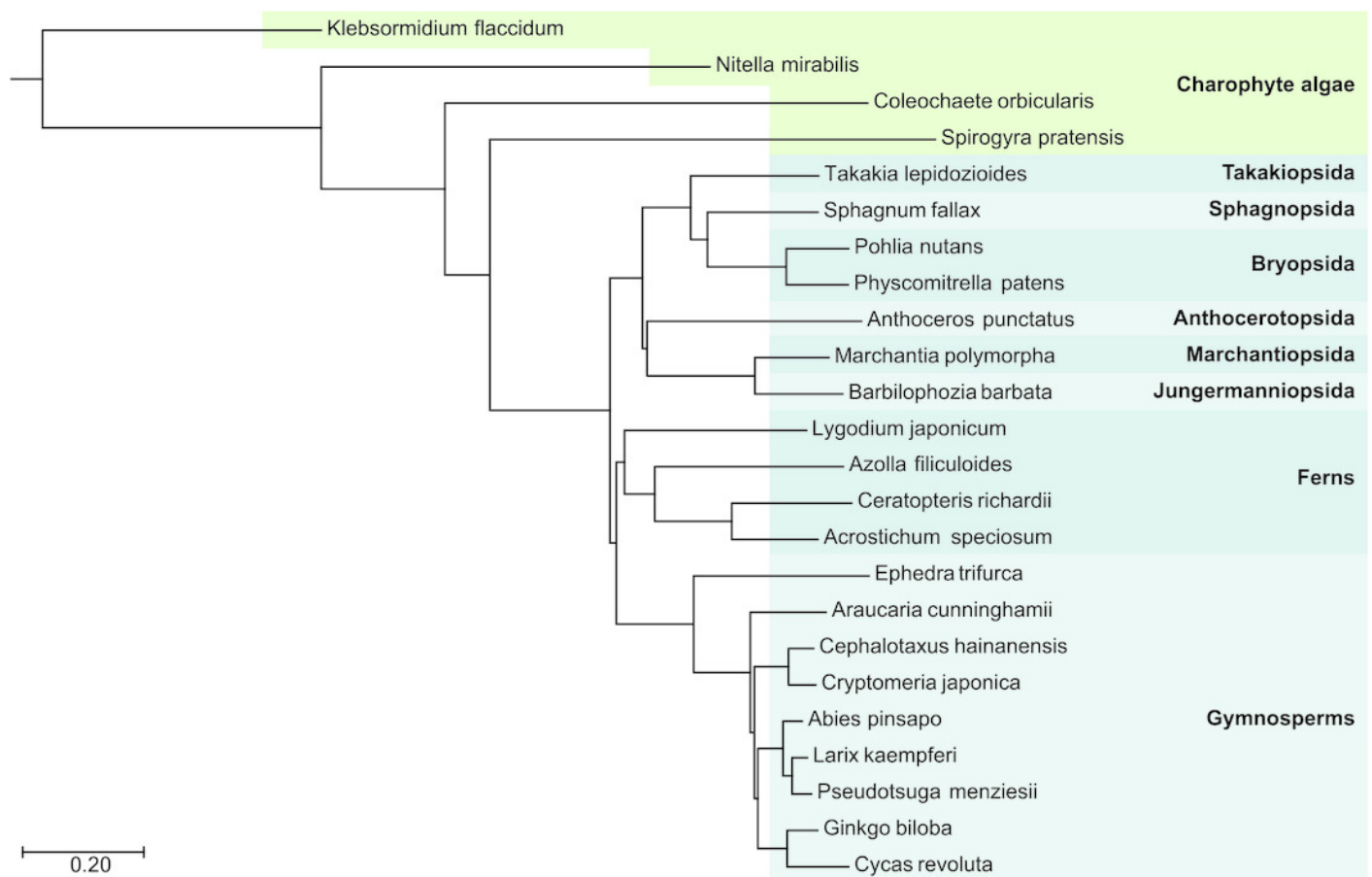
Query 860 TCCTGAGCTA 869
          |||
Sbjct 1135 TCCTGAGCTA 1144

```

## Figure 10

The phylogenetic tree based on sequence alignment of the conserved region of SGS3 amino acid sequences in selected lower plant species.

Neighbor-joining tree was obtained at <http://www.ncbi.nlm.nih.gov/tools/cobalt/> with the use of default parameters. The scale bar denotes the estimated number of amino acid substitutions per site. *K. flaccidum* was used as outgroup.





**Table 1** (on next page)

List of the putative TAS3 loci in Sphagnopsida and Takakiopsida

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**Table 1.**  
**List of the putative TAS3 loci in Sphagnopsida and Takakiopsida**

Plant species	Locus name	Subgenus	Length	Sequence source
<i>Sphagnum angustifolium</i>	Sphan-285	<i>Cuspidata</i>	285 nts	MF682529
<i>S. girgensohnii</i>	Sphgi-292	<i>Acutifolia</i>	292 nts	MF682530
<i>S. fallax</i>	contig super_37	<i>Cuspidata</i>	285 nts	SRX2120232
<i>S. fallax</i>	Sphfalx0293s0011	<i>Cuspidata</i>	277 nts	Sphfalx0293s0011*
<i>S. recurvum</i>	Sphre-283	<i>Cuspidata</i>	283 nts	SRX1513231
<i>S. recurvum</i>	Sphre-277	<i>Cuspidata</i>	277 nts	SRX1513231
<i>S. magellanicum</i>	Sphma-285	<i>Sphagnum</i>	285 nts	SRX2330962
<i>S. magellanicum</i>	Sphma-286	<i>Sphagnum</i>	286 nts	SRX2330962
<i>S. palustre</i>	Sphpa	<i>Sphagnum</i>	partial	SRX1516347
<i>S. cribrosum</i>	Sphcri	<i>Subsecunda</i>	291 nts	ERX443237
<i>S. lescurii</i>	Sphle	<i>Subsecunda</i>	partial	ERX337183
<i>Takakia lepidozoides</i>	Takle-207	Not applicable	207 nts	ERX2100030 SKQD-2076588**

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\* - PHYTOZOME accession; \*\* - 1KP accession (Xia et al., 2017). Different sphagnum subgenera are colored specifically.

**Table 2** (on next page)

List of the putative TAS3 loci in Anthocerotophyta and Marchantiophyta

1 **Table 2.**  
 2 **List of the putative TAS3 loci in Anthocerotophyta and Marchantiophyta**  
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Plant species	Class/subclass	Order	Length	Sequence source
<i>Folioceros fuciformis</i>	Anthocerotopsida/Anthocerotidae	Anthocerotales	244 nts	SRS2162762
<i>Marchantia polymorpha</i> 1-Mpo	Marchantiopsida/Marchantiidae	Marchantiales	256 nts	KC812742
<i>Marchantia emarginata</i>	Marchantiopsida/Marchantiidae	Marchantiales	262 nts	SRX1952816
<i>Conocephalum japonicum</i>	Marchantiopsida/Marchantiidae	Marchantiales	252 nts	SRX1952810
<i>Ricciocarpos natans</i>	Marchantiopsida/Marchantiidae	Marchantiales	235 nts	ERX337127
<i>Dumortiera hirsuta</i>	Marchantiopsida/Marchantiidae	Marchantiales	243 nts	SRX1126014
<i>Plagiochasma appendiculatum</i>	Marchantiopsida/Marchantiidae	Marchantiales	247 nts	SRX1741567
<i>Conocephalum conicum</i>	Marchantiopsida/Marchantiidae	Marchantiales	248 nts	ILBQ_2006554*
<i>Lunularia cruciata</i>	Marchantiopsida/Marchantiidae	Lunulariales	220 nts	TXVB_2071521*
<i>Marchantia paleacea</i>	Marchantiopsida/Marchantiidae	Marchantiales	257 nts	HMHL_2051051*
<i>Metzgeria crassipilis</i>	Jungermannopsida/Metzgeriidae	Metzgeriales	226 nts	ERX337128
<i>Pellia endiviifolia</i>	Jungermannopsida/Pelliidae	Pelliales	192 nts	SRX726500

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 5 \* - 1KP accession (Xia et al., 2017).

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**Table 3** (on next page)

List of the putative TAS6/TAS3 loci of Bryophyta in transcribed sequences found in 1KP database

1 **Table 3.**  
 2 **List of the putative TAS6/TAS3 loci of Bryophyta in transcribed sequences found in 1KP**  
 3 **database**  
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Plant species	Class/subclass	Order	Length* and type	Sequence source
<i>Timmia austriaca</i>	Bryopsida/Timmiidae	Timmiales	TAS6/TAS3 (874nts)	ZQRI-2061439 ZQRI-2063082
<i>Thuidium delicatulum</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (837nts)	EEMJ-2003175
<i>Hypnum subimponens</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (823nts)	LNSF-2068452
<i>Pseudotaxiphyllum elegans</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (1590nts)	QKQO-2009669
<i>Anomodon attenuatus</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (843nts)	QMWB-2059873
<i>Anomodon rostratus</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (829nts)	VBMM-2003482
<i>Schwetschkeopsis fabronia</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (854nts)	IGUH-2166854
<i>Leucodon sciuroides</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (852nts)	ZACW-2016434
<i>Fontinalis antipyretica</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (1410nts)	DHWX-2007057
<i>Rhytidiadelphus loreus</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (830nts)	WSPM-2009782
<i>Rhynchostegium serrulatum</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (853nts)	JADL-2047695
<i>Climacium dendroides</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (809nts)	MIRS-2012325
<i>Calliargon cordifolium</i>	Bryopsida/Bryidae	Hypnales	TAS6 (95nts)	TAVP-2006322
<i>Neckera douglasii</i>	Bryopsida/Bryidae	Hypnales	TAS6/TAS3 (839nts)	TMAJ-2023603
<i>Plagiomnium insigne</i>	Bryopsida/Bryidae	Bryales	TAS6/TAS3 (914nts)	BGXB-2010105
<i>Orthotrichum lyellii</i>	Bryopsida/Bryidae	Orthotrichales	TAS6 (192nts)	CMEQ-2080784
<i>Hedwigia ciliata</i>	Bryopsida/Bryidae	Hedwigiales	TAS6/TAS3 (877nts)	YWNF-2050742
<i>Philonotis fontana</i>	Bryopsida/Bryidae	Bartramiales	TAS6/TAS3 (893nts)	ORKS-2058791
<i>Aulacomnium heterostichum</i>	Bryopsida/Bryidae	Rhizogoniales	TAS6/TAS3 (863nts)	WNGH-2088134
<i>Scouleria aquatic</i>	Bryopsida/Dicranidae	Scouleriales	TAS6/TAS3 (partial)	BPSG-2088977
<i>Syntrichia princeps</i>	Bryopsida/Dicranidae	Pottiales	TAS6/TAS3 (partial)	GRKU-2074985
<i>Leucobryum glaucum</i>	Bryopsida/Dicranidae	Dicranales	TAS6/TAS3 (763nts)	RGKI-2062694
<i>Leucobryum albidum</i>	Bryopsida/Dicranidae	Dicranales	TAS6/TAS3 (763nts)	VMXJ-2128109
<i>Dicranum scoparium</i>	Bryopsida/Dicranidae	Dicranales	TAS6 (105nts)	NGTD-2092412

<i>Ceratodon purpureus</i>	Bryopsida/Dicranidae	Pseudoditrichales	TAS6/TAS3 (1121nts)	FFPD-2005850 SRX2065999
<i>Racomitrium varium</i>	Bryopsida/Dicranidae	Grimmiales	TAS6/TAS3 (724nts)	RDOO-2117129
<i>Physcomitrium sp.</i>	Bryopsida/Funariidae	Funariales	TAS6 (partial)	YEPO-2071108
<i>Physcomitrium sp.</i>	Bryopsida/Funariidae	Funariales	TAS6 (178nts)	YEPO-2000016
<i>Physcomitrium sp.</i>	Bryopsida/Funariidae	Funariales	TAS6/TAS3 (821nts)	YEPO-2016361
<i>Encalypta streptocarpa</i>	Bryopsida/Funariidae	Encalyptales	TAS6/TAS3 (883nts)	KEFD-2058811
<i>Diphyscium foliosum</i>	Bryopsida/Diphysciidae	Diphyscales	TAS6/TAS3 (832nts)	AWOI-2069791
<i>Tetraphis pellucida</i>	Tetraphidopsida	Tetraphidales	TAS6 (partial)	HVBQ-2112923
<i>Atrichum angustatum</i>	Polytrichopsida	Polytrichales	TAS6/TAS3 (810nts)	ZTHV-2082998
<i>Andreaea rupestris</i>	Andreaeopsida	Andreaeales	TAS6/TAS3 (869nts)	WOGB-2010369
<i>Takakia lepidozioides</i>	Takakiopsida	Takakiales	TAS6/TAS3 (1040nts)	SKQD-2076588

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6 \* - The length indicates total size of TAS6-TAS3 complex element (from the 5' miR529 target  
7 site in TAS6 to 3' miR390 target site in TAS3) or isolated TAS6 (between miR529 and miR156  
8 target sites).

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