

ON THE SYSTEMATIC POSITION OF THE GENUS SASAOKAEA (BRYOPHYTA)  
О СИСТЕМАТИЧЕСКОМ ПОЛОЖЕНИИ РОДА SASAOKAEA (BRYOPHYTA)

M.S. IGNATOV<sup>1</sup> & I.A. MILYUTINA<sup>2</sup>

М.С. ИГНАТОВ<sup>1</sup>, И.А. МИЛЮТИНА<sup>2</sup>

Abstract

The monospecific East Asian genus *Sasaokaea* was traditionally considered as a member of the Amblystegiaceae, or sometimes Neckeraceae. Analysis of nr ITS and chloroplastic *trnL*-F data both indicate its position in the Leskeaceae. The morphological characters supporting this placement are discussed.

Резюме

Восточноазиатский монотипный род *Sasaokaea* обычно относили к семейству Amblystegiaceae, иногда к Neckeraceae. Анализ ДНК ядерной ITS и хлоропластной *trnL*-F в обоих случаях указывает на положение рода в Leskeaceae. Обсуждаются морфологические признаки, согласующиеся с таким положением рода.

KEYWORDS: Amblystegiaceae, ITS, Leskeaceae, mosses, *Sasaokaea*, *trnL*-F

INTRODUCTION

The splitting of the large family Amblystegiaceae into Calliergonaceae and Amblystegiaceae (Vanderpoorten et al., 2002) or even further with the segregation of the Scorpidiaceae (Ignatov et al., 2007) left some small genera outside of analysis, mostly due to unavailability of fresh material for sequencing. Among others remains the genus *Sasaokaea* that includes the only species, *S. aomoriensis* (Paris) Kanda, known in Japan and in a single locality in Far East Russia. Originally this species was described in the genus *Hypnum* that in the beginning of XX century included representatives of many genera and families of pleurocarps. Shortly after that it was classified in *Drepanocladus* (Brotherus, 1908), in the Amblystegiaceae. However later, Brotherus (1929) described another species in a new monotypic genus, *Sasaokaea japonica* Broth. in the Neckeraceae. Noguchi (1939) synonymized *Sasaokaea japonica* with *Drepanocladus aomoriensis*, and then, in revision of Japanese Amblystegiaceae, Kanda

(1976) accepted this species and also the genus *Sasaokaea*, mentioning a number of important characters differentiating these genera.

Having long narrow paraphyllia one might presume the closest affinity to *Palustriella*. However Ochyra (1988), who revised this complex of genera, excluded *Sasaokaea* from Amblystegiaceae and again suggested its position in Neckeraceae, close to *Metaneckera*. The latter suggestion met no wide acceptance and most of recent authors accepted *Sasaokaea* within Amblystegiaceae (<http://www.tropicos.org>; Goffinet et al., 2009; Iwatsuki, 2004; Noguchi, 1991).

As the molecular phylogenetic methods provide a powerful tool for checking affinities of plants, including pleurocarpous mosses, a small analysis was undertaken to resolve this problem using our previous and GenBank data.

Nuclear ITS, as one of the most polymorphous markers, was used in this study, as well as *trnL*-F intron, as their independent analysis could be a double evidence for the position of the taxon in question.

<sup>1</sup> – Main Botanical Garden of Russian Academy of Sciences, Botanicheskaya, 4, Moscow 127276 Russia – Россия 127276 Москва, Ботаническая, 4, Главный ботанический сад им. Н.В.Цицина РАН; e-mail: misha\_ignatov@list.ru

<sup>2</sup> – A.N.Belozersky' Research Institute of Physico-Chemical Biology, Moscow State University, Moscow 119991 Russia – Россия 119991 Москва, МГУ, НИИ Физико-химической биологии им. А.Н. Белозерского; e-mail: iramilyutina@yandex.ru



## MATERIAL AND METHODS

*Sasaokaea* was studied by specimen from S: “Honshu, Aichi-ken, Minami-shidara-gun, Tsukude-mura, Iwanami, 450 m a.s.l. In bog. N. Takaki, 1974-09-28; Musci Japonici exsiccati nr. 1314”. Its sequences of nr ITS and chloroplastic *trnL-F* were analyzed with the data from a previous analysis of pleurocarps (Ignatov et al., 2007). This previous set was reduced, so mostly taxa with paraphyllia or their closest relatives were retained, as they were considered possibly related to *Sasaokaea*. Specimen vouchers and GenBank accessions are in Table 1.

The laboratory protocol was essentially the same as in some of our previous analyses (e.g. Gardiner et al., 2005). Maximum parsimony analysis was performed in Nona (Goloboff, 1994) under Winclada shell (Nixon, 1999). Jackknife support was calculated for 2000 iterations (N searches 10, hold 10, max trees 100, Do max).

## RESULTS

Jackknife ITS tree is not well resolved, although the support of clades with well-known representatives of the Amblystegiaceae (100), Pseudoleskeaceae (92), Pseudoleskeellaceae (99), etc. is high. A biggest clade that includes representatives of Neckeraaceae, Climaciaceae, Hylocomiaceae, Pseudoleskeaceae, Anomodontaceae got a low support of 51. *Sasaokaea* was found in a clade with the Leskeaceae (excluding the Pseudoleskeellaceae and *Lindbergia* s.l.) plus the Thuidiaceae, with a rather high support of 83. Within this clade it is in a basal unresolved polytomy, with genera *Actinothuidium*, *Boulaya*, *Helodium*, *Raiiella*, whereas this clade includes also *Thuidium* s. str. (5 species, with high support, 100) and one another clade, *Leskea*+*Haplocladium*+*Pseudoleskeopsis zippelii* (6 species, with moderate support, 65).

Jackknife analysis for *trnL-F* data supports almost no clades and is not shown here, but the strict consensus tree (Fig. 2) found clades similar to that in ITS analysis. *Sasaokaea* was found in a clade with *Boulaya*, *Haplocladium*, *Helodium*, *Leskea*, *Pseudoleskeopsis*, *Raiiella*, and *Thuidium*.

## DISCUSSION

The topologies from analyses of both nuclear and chloroplastic genomes clearly indicate position of *Sasaokaea* in the Leskeaceae, in case of ITS with a sufficient statistical support.

As far as we know, the idea of such a place-

ment of subaquatic *Sasaokaea* into a mostly xerophytic family has never been suggested. The specific character of Leskeaceae+Thuidiaceae is the presence of branched paraphyllia, a rare character in pleurocarps, known in only one another group of Climaciaceae+Hylocomiaceae.

There is a specific pattern in the Climaciaceae where paraphyllia are arranged in longitudinal rows and likely not homologous to paraphyllia in other groups of pleurocarps (Norris & Ignatov, 2000). In the Hylocomiaceae paraphyllia when present are very dense up to ultimate branches, obscuring the understanding of their homology, although likely having the same origin as in the Climaciaceae, i.e. without tendency to concentrate around branch initials. The latter is obvious in the Leskeaceae (*Leskea*, *Haplocladium*, etc.), while in *Thuidium* this pattern can be seen on the secondary branches (as on the stem paraphyllia are too dense). In *Sasaokaea* paraphyllia are dense on the stem, but scattered on branches, where their position next to branch initials is obvious (Figs. 3, 4: 1-4), identical to the Leskeaceae–Thuidiaceae pattern, which supports this new placement of *Sasaokaea*.

*Metaneckera* is another genus that was considered related to *Sasaokaea*. It also has paraphyllia concentrated around branch initials. However paraphyllia in *Metaneckera* are unbranched, very dense on the stem and moderately dense in ultimate branches, where the specific pattern occur: paraphyllia are clustered between neighboring leaf corners (Figs. 4: 5-6).

## ACKNOWLEDGEMENTS

We are grateful to Lars Hedenäs for the permission to study DNA from S specimen and to Anna Ivanova for improving English. The work was partly supported by RFBR 10-04-00678 and Biodiversity Program of Russian Academy of Sciences.

## LITERATURE CITED

- BROTHERUS, V.F. 1908. Musci (Laubmoose). 2. Pleurocarpi. – In: Engler, A. & Prantl, K. (eds.), *Die Natürlichen Pflanzenfamilien*, Bd. 1(3), W. Engelmann, Leipzig, pp. 1009-1152.
- BROTHERUS, V.F. 1929. Musci novi asiatici. – *Rev. Bryol.*, n.s. 2: 1-16.
- GARDINER, A., M. IGNATOV, S. HUTTUNEN & A. TROITSKY 2005. On resurrection of the families Pseudoleskeaceae Schimp. and Pylaisiaceae Schimp. (Musci, Hypnales). – *Taxon* 54: 651-663.
- GOFFINET, B., W.R. BUCK & A.J. SHAW 2009 [2008]. Morphology, anatomy, and classification of the Bryophyta. – In:

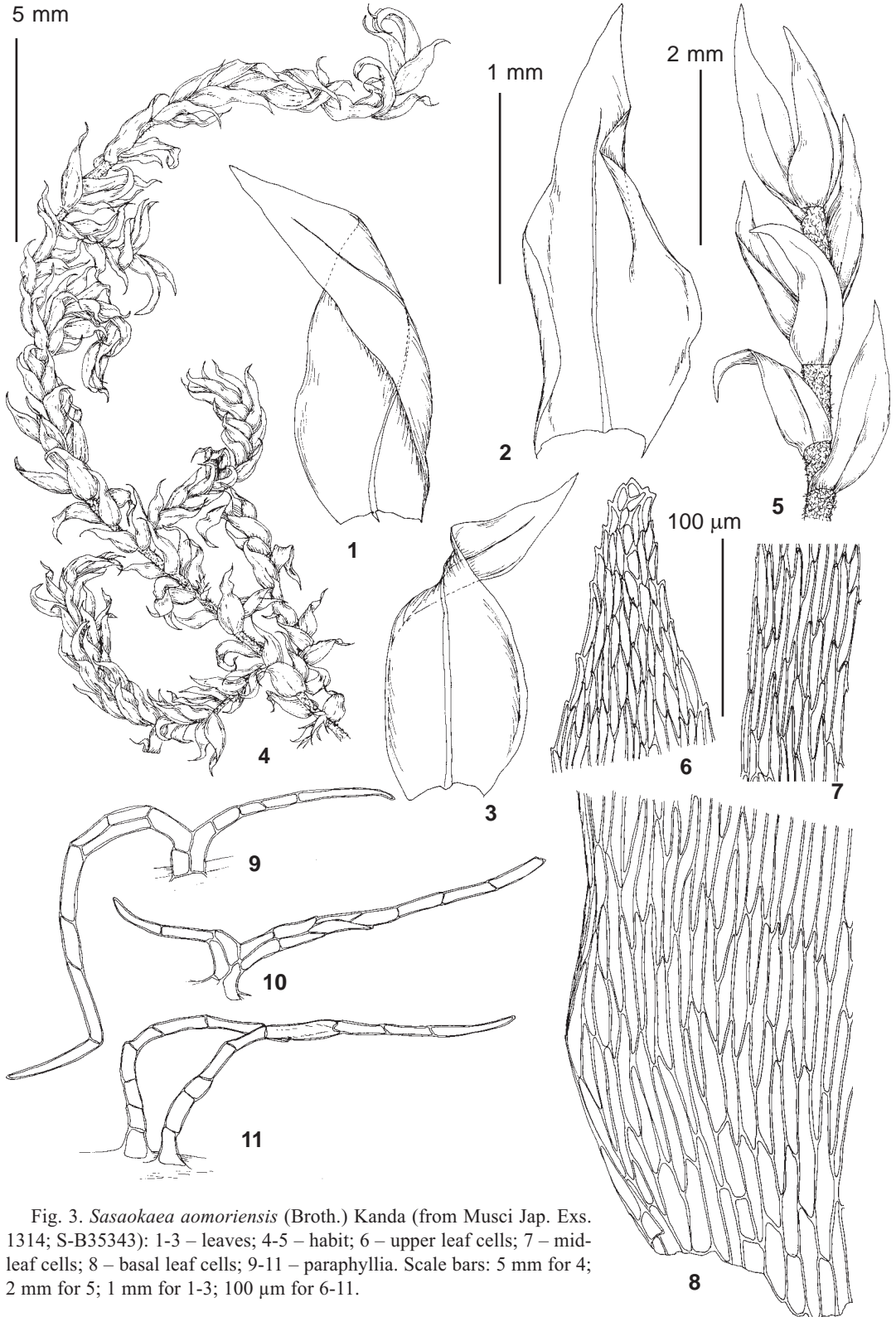


Fig. 3. *Sasaokaea aomoriensis* (Broth.) Kanda (from Musci Jap. Exs. 1314; S-B35343): 1-3 – leaves; 4-5 – habit; 6 – upper leaf cells; 7 – mid-leaf cells; 8 – basal leaf cells; 9-11 – paraphyllia. Scale bars: 5 mm for 4; 2 mm for 5; 1 mm for 1-3; 100 µm for 6-11.

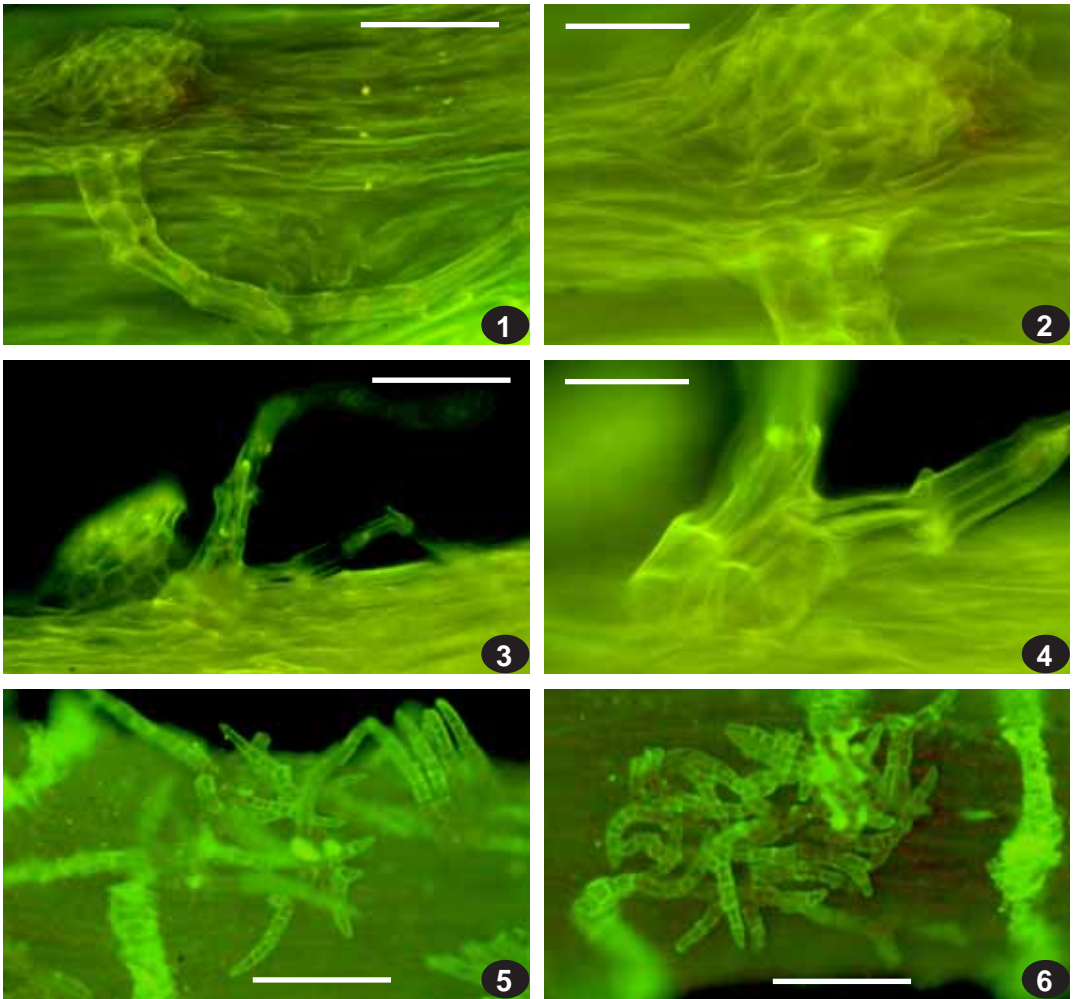


Fig. 4. 1-4: *Sasaokaea aomoriensis* (Musci Japonici exsiccati, 1314): paraphyllium beside branch initial; 5-6: *Metaneckera menziesii* (from: California, August 1989, Ignatov, MHA): paraphyllia on secondary branch, showing their dense arrangement between corners of neighboring leaves. Scale bars: 100  $\mu$ m for 1, 3, 5-6; 20  $\mu$ m for 2, 4.

Goffinet, B. & A. J. Shaw (eds.) *Bryophyte Biology*, 2d ed. Cambridge University Press: Cambridge: 55-138.

GOLOBOFF, P.A. 1994. NONA: A Tree Searching Program. – Program and documentation. Argentina, Tucumán, published by the author.

IGNATOV, M., A. GARDINER, V. BOBROVA, I. MILYUTINA, S. HUTTUNEN & A. TROITSKY 2007. On the relationships of mosses of the order Hypnales, with special reference to taxa traditionally classified in the Leskeaceae. – In: Newton, A.E. & R. Tangney (eds.), *Pleurocarpus mosses: systematics and evolution*. CRC Press, Boca Rota–London–New York: 177-213.

IWATSUKI, Z. 2004. New catalog of the mosses of Japan. – *J. Hattori Bot. Lab.* **96**: 1-182.

KANDA, H. 1976 [1978]. A revision of the family Amblystegiaceae of Japan. 2. – *J. Sci. Hiroshima Univ., ser. B, Div. 2*, **16**: 47-119.

NIXON, K.C. 1999. *Winclada* (BETA) ver. 0.9.9. available at

[http://www.cladistics.com/about\\_winc.html](http://www.cladistics.com/about_winc.html).

NOGUCHI, A. 1939. Notes on Japanese Musci. 3. – *J. Jap. Bot.* **15**: 754-766.

NOGUCHI, A. 1991. Illustrated moss flora of Japan. Pt. 4. – *Hattori Botanical Laboratory, Nichinan*: 743-1012.

NORRIS, D.H. & M.S. IGNATOV 2000. Observations on stem surface anatomy in *Climacium* and *Pleuroziopsis* (Climaciaceae, Musci). – *Arctoa* **9**: 151-154.

OCHYRA, R. 1988. The family placement of *Sasaokaea* Broth. and *Crassicosta* Crum & Sharp (Musci). – In T. Herben (ed.) *Abstracts of Sixth Meeting of the Central and East European Bryological Working Group*: 9.

VANDERPOORTEN, A., L. HEDENÄS, C.J. COX & A.J. SHAW 2002. Circumscription, classification, and taxonomy of the Amblystegiaceae (Bryopsida) inferred from nuclear and chloroplast DNA sequence data and morphology. – *Taxon* **51**: 115-122.

Table 1. Specimens used in molecular phylogenetic analysis with GenBank accession numbers

Species	<i>trnL-F</i>	ITS1	ITS2
<i>Abietinella abietina</i> (Hedw.) M. Fleisch. 1	AY009850	AY009802	AY009802
<i>Abietinella abietina</i> (Hedw.) M. Fleisch. 2		AJ417494	AJ417494
<i>Actinotuidium hookeri</i> (Mitt.) Broth.		AY568547	AY568547
<i>Amblystegium serpens</i> (Hedw.) Bruch et al.	AY009827	AF168152	AF168152
<i>Anomodon attenuatus</i> (Hedw.) Hueb.		JF280973	AJ277231
<i>Anomodon rugelii</i> (Müll. Hal.) Keissl.	AF161116	AJ288420	AJ277232
<i>Boulaya mittenii</i> (Broth.) Card.	AM990347	FM161080	FM161080
<i>Climacium americanum</i> Brid.	AF161158		
<i>Climacium dendroides</i> (Hedw.) F. Weber & D. Mohr		AJ288355	AJ288569
<i>Cratoneuron filicinum</i> (Hedw.) Spruce	AY009817	AY009812	AY009812
<i>Drepanocladus aduncus</i> (Hedw.) Warnst.		AF180949	AF180949
<i>Echinodium umbrosum</i> (Mitt.) A. Jaeger		AY999172	AY999172
<i>Haplocladium angustifolium</i> (Hampe & Müll. Hal.) Broth.	AY527129	AY528884	AY528885
<i>Haplocladium virginianum</i> (Brid.) Broth.		AF168160	AF168160
<i>Haplohymenium triste</i> (Cesati) Kindb.		AY568551	AY568551
<i>Helodium blandowii</i> (F. Weber & D. Mohr) Warnst.	AY009852	AY009803	AY009803
<i>Heterocladium procurrens</i> (Mitt.) A. Jaeger		AY695756	AY695782
<i>Heterocladium dimorphum</i> (Brid.) Bruch et al.		AY695757	AY695771
<i>Hylocomium splendens</i> (Hedw.) Bruch et al.	AF152385	AJ288336	AJ270021
<i>Isopterygiopsis muelleriana</i> (Schimp.) Z. Iwats.	AY527138	AY528882	AY528882
<i>Lescuraea incurvata</i> (Hedw.) Lawt.		AY693661	AY693661
<i>Lescuraea patens</i> (Lindb.) Arnell & C. E. O. Jensen		AY695746	AF516149
<i>Lescuraea secunda</i> Arnell		AF516164	AF516150
<i>Lescuraea saxicola</i> (Bruch et al.) Mol.	AY683569		
<i>Lescuraea plicata</i> (Schleich. ex F. Weber & D. Mohr) Broth.	AY683596	AY695740	AY695765
<i>Leskea gracilescens</i> Hedw.		AF176277	AF176277
<i>Leskea polycarpa</i> Hedw.	AY527134	AY528889	AF516151
<i>Scorpidium revolvens</i> (Sw. ex Anonymo) Rubers		AF168140	AF168140
<i>Lindbergia brachyptera</i> (Mitt.) Kindb. 1	AY683571	AY695760	AY695763
<i>Lindbergia brachyptera</i> (Mitt.) Kindb. 2		FM161151	FM161151
<i>Lindbergia grandiretis</i> (Lindb. ex Broth.) Ignatov & Ignatova 1		JF280965	JF280965
<i>Lindbergia grandiretis</i> (Lindb. ex Broth.) Ignatov & Ignatova 2		JF280966	JF280966
<i>Lindbergia patentifolia</i> Dixon	JF280980	JF280969	JF280969
<i>Lindbergia sinensis</i> (Müll. Hal.) Broth. 1		AF516170	AF516153
<i>Lindbergia sinensis</i> (Müll. Hal.) Broth. 2		JF280970	JF280970
<i>Loeskeobryum brevirostre</i> (Brid.) M. Fleisch.	AF161172	JF280975	AJ270022
<i>Mamillariella geniculata</i> Laz.		AY693652	AY693652
<i>Miyabaea rotundifolia</i> Cardot		FM161155	FM161155
<i>Neckera besseri</i> (Lob.) Jur.	AF543543	JF280972	AF543544
<i>Neckera pennata</i> Hedw.	AF315072	AY009809	AY009809
<i>Palustriella falcata</i> (Brid.) Hedenäs		AF168158	AF168158
<i>Pseudoleskeella nervosa</i> (Brid.) Loeske	AY527135	AF516167	AF516152
<i>Pseudoleskeella catenulata</i> (Brid. ex Schrad.) Kindb.	AY683578	AY695747	AF516154
<i>Pseudoleskeella papillosa</i> (Lindb.) Kindb.		AY695753	AY695784
<i>Pseudoleskeella tectorum</i> (Funck ex Brid.) Kindb. ex Broth.		AF516168	AY695776
<i>Pseudoleskeopsis imbricata</i> (Hook. & Wilson) Thér.	AY683581	AY693653	AY693653
<i>Pseudoleskeopsis zippelii</i> (Dozy & Molk.) Broth. 1		FM161206	FM161206
<i>Pseudoleskeopsis zippelii</i> (Dozy & Molk.) Broth. 2		AY568548	AY568548
<i>Rauvolfia fujisana</i> (Paris) Reimers		AY568546	AY568546
<i>Rigodiadelphus robustus</i> (Lindb.) Nog.		AF516166	AF516156
<i>Sanionia uncinata</i> (Hedw.) Loeske	AY009860	AF168148	AF168148
<i>Sasaokaia aomoriensis</i> (Paris) Kanda	JF280981	JF280974	JF280974
<i>Scorpidium scorpioides</i> (Hedw.) Limpr.	AY009791	AY009790	AY009790
<i>Thamnobryum alopecuroides</i> (Hedw.) Nieuwl. ex Gang.		FM161218	FM161218
<i>Thelia asprella</i> (Schimp.) Sull. & Lesq.		AJ288413	AJ277225
<i>Thuidium cymbifolium</i> (Dozy et Molk.) Dozy & Molk.		AY568542	AY568542
<i>Thuidium kanedae</i> Sakurai		AY568541	AY568541
<i>Thuidium glaucinoides</i> Broth.		AY568544	AY568544
<i>Thuidium pristocalyx</i> (Müll. Hal.) A. Jaeger		AY568540	AY568540
<i>Thuidium delicatulum</i> (Hedw.) Bruch et al.	AF161132		
<i>Thuidium tamariscinum</i> (Hedw.) Bruch et al.	AF023770		
<i>Thuidium thermophilum</i> Czernyadjeva	EF368012	EF368013	EF368013