**Abstract**

Pseudotaxiphyllum subfalcatum, a species previously known only from North and South America, is found in herbarium collections from Asiatic Russia. Its identity is confirmed by molecular markers, and sequence data from GenBank also indicate the occurrence of this species in Japan. The description and illustration of Russian specimens are provided and a comparison of the three Pseudotaxiphyllum species currently known in Russia is given. Pseudotaxiphyllum elegans occurs in NW European Russia, Caucasus, Yakutia, and the Northern and Southern Far East; P. distichaceum grows in the Kuril Islands, and P. subfalcatum is found in Iturup Island (South Kuril Islands) and in the Baikal area of southern Siberia.

**Keywords:** phytogeography, disjunctions, mosses, molecular phylogeny, ITS, trnL–F

**INTRODUCTION**

The genus Pseudotaxiphyllum Z. Iwats. was segregated from Isopterygium Mitt. to accommodate species mainly with axillary propagules, lacking pseudoparaphyllia and having differentiated annuli (Iwatsuki, 1987). Seven species were listed by Iwatuki (l.c.), i.e. P. arquifolium (Bosch & Sande Lac.) Z. Iwats., P. pohliaecarpum (Sull. & Lesq.) Z. Iwats., P. densum (Cardot) Z. Iwats., P. distichaceum (Mitt.) Z. Iwats., P. elegans (Brid.) Z. Iwats., P. fauriei (Cardot) Z. Iwats., and P. maebarae (Sakurai) Z. Iwats. Later two American species, P. homomallifolium (Redf.) Ireland and P. richardssii (E.B. Bartram) Ireland, were added to the genus by Ireland (1991). Ireland (l.c.), also submerged Isopterygium s. a. Jaeger (described from the U.S.A. as Plagiothecium subfalcatum Austin) into synonymy with P. distichaceum (described from the Himalayas), thus extending the distribution of P. distichaceum into North America.

Subsequently, P. laetevirens (Dixon & Luisier ex F. Koppe & Düll) Hedenäs from Macaronesia was recognized at the species level (Hedenäs, 1992); P. falcifolium (Hook. f. & Wilson) S. He from New Zealand was transferred to the genus from Homalia (He, 1997); and P. obtusifolium Z. Iwats. & B.C. Tan from Taiwan and mainland China was described as new to science (Iwatsuki & Tan, 2004). Pseudotaxiphyllum is currently treated as a member of Plagiotheciaceae based on molecular-phylogenetic data (Huttunen et al., 2013).
Phylogenetic relationships and species delimitation within *Pseudotaxiphyllum* were addressed by Li et al. (2015). Their study of chloroplast and nuclear molecular markers and morphological characters of mainly East Asian specimens, with addition of some North American and European samples, resulted in the recognition of eight species within the genus. Four species listed by Iwatsuki (1987), i.e. *P. aeguifolium*, *P. densum*, *P. fauriei*, and *P. pohliaecarpum* were synonymized with *P. distichaceum*. At the same time, Li et al. (2015) distinguished the North American specimens referred to *P. distichaceum* from the East Asian collections of this species, referring the American plants to *P. subfalcatum* (Austin) X.Q. Li, Q. Zuo & Y.F. Wang.

Until recently, only one species of *Pseudotaxiphyllum*, *P. elegans*, was known from the territory of Russia: it was reported from NW European Russia, Caucasus, Chukotka, Kamchatka, Magadan Province and Primorsky Territory (Ignatova et al., 2006) and subsequently found in Yakutia (Ivanova et al., 2018). One more species, *P. pohliaecarpum*, was reported from the Kuril Islands (Bakalin et al., 2009).

During the identification of unnamed herbarium collections kept in IRK, OA found one rather scarce specimen of *Pseudotaxiphyllum* from the Republic of Buryatia, collected in 2011 by SK, in the vicinity of Lake Baikal. Morphological characters of these plants, i.e. flattened foliage, cultriform leaves and propagules conspicuously clustered at the shoot tips, were in agreement with the circum-scription of the North American plants of “*P. distichaceum*” (Ireland, 2014). The propagula shape (vermiform, 2-3 cells wide, with leaflets at the tips) also supported this circumscription of the North American plants of "*P. distichaceum*" (Ireland, 2014). The propagula shape (vermiform, 2-3 cells wide, with leaflets at the tips) also supported this identification. Since this specimen could be the first record of the American *P. subfalcatum* in Eurasia, testing with molecular markers was undertaken, which confirmed the identity of the Baikal specimen with the North American *P. subfalcatum*. However, in the course of this search, we also revealed one sample from Japan with the trnL–F sequence stored in GenBank, which is identical to *P. subfalcatum*, but not presented in the study of Li et al. (2015).

Thus the total revision of Far Eastern *Pseudotaxiphyllum* herbarium collections was undertaken, with sequencing several samples, in order to elucidate the species composition of this genus in Russia. In species circumscription we follow Li et al. (2015).

**Material and Methods**

*Pseudotaxiphyllum* specimens from Russia were studied for nuclear ITS and chloroplastic trnL–F, as these markers were proved to be the most informative in pleurocapous mosses (cf. Huttunen et al., 2013), and moreover studied in many specimens of *Pseudotaxiphyllum* from Asia (Li et al., 2015).

Total genomic DNA was extracted from dry plants using the Nucleospin Plant Extraction Kit (Macherey-Nagel, Germany). Laboratory protocol was essentially the same as in previous moss studies, described in detail by, e.g., Gardiner et al. (2005).

Sequences were embedded in GenBank data, mostly from the Li et al. (2015) analysis. We selected specimens where both ITS and trnL–F were available, omitting mostly those where only one locus was known. One exception was made for a Japanese specimen where only trnL–F was known: it was included into the analyses due to its phytogeographical importance, as explained below in the Discussion section. Sequences where aligned by Clustal and modified manually using BioEdit 7.0 (Hall, 1999). Bayesian analysis of the ITS dataset was conducted in MrBayes (Huelsenbeck & Ronquist, 2001) using the GTR+G model. It was run for 20,000,000 generations with sampling every 1000 generations, the chain temperature was set at 0.02. The first 25% of sampled trees were discarded for the burn-in.

**Results**

The molecular phylogenetic tree resolved *Pseudotaxiphyllum elegans* in a maximally supported clade sister to the rest of the genus, which has a rather complicated phylogeny. The latter clade has low support (PP=0.74) and is composed of two maximally supported clades. One includes only two specimens from SE China (Zhejiang and Taiwan), referred by Li et al. (2015) to *P. distichaceum*, and in the tree we use this name with the addition ‘s.l.’. Another clade performs the grade of (1) subclade of *P. subfalcatum* s.l.; (2) subclade of *P. mayebarae*; and terminal clade (3) of *P. distichaceum* (note that in GenBank, *P. distichaceum* specimens are deposited under the name *P. pohliicarpum*). Subclade of *P. subfalcatum* s.l. (1) included one specimen from Japan, deposited in GenBank under the name *P. pohliaecarpum* (“*P. pohliicarpum*”). Subclade 3 includes several nested clades with various supports, from low to maximal.

**Discussion**

The obtained phylogeny is based largely on specimens used in the Li et al. (2015) analysis, thus the overall tree topology is almost the same and that thoroughly discussed already in that publication. We repeat that the species *P. distichaceum* in the present circumscription likely comprises a complex of cryptic species; however, it requires an additional study, as attempts of Li et al. (2015) to find morphological differences did not succeed. The distinct position of specimens from Zhejiang and Taiwan is especially enigmatic.

Specimens from Baikal Lake area and Iturip Island were found in the clade with *P. subfalcatum*, and moreover they are subidentical with a specimen of *P. subfalcatum* from North Carolina, U.S.A. Two other American specimens, from Maine and Pennsylvania, are slightly different molecularly, although they have no apparent morphological differences from the North Carolina specimen (according to their comparison by Allen and Atwood, letter of 16 October 2019). Japanese specimen QZJP85 (“*P. pohliicarpum*” in Fig. 1, GenBank accession HQ665374) has only trnL–F sequence, but it was resolved in *P. subfalcatum*-clade, because it has three
**Pseudotaxiphyllum subfalcatum new to Asia**

Figure 1. Bayesian tree of *Pseudotaxiphyllum* inferred from nuclear ITS and chloroplastic *trn*L–F sequences. Names are given according to Li *et al.* (2015) and partly differ from names in Genbank. New sequence data are in Appendix 1. Posterior probabilities >0.70 are shown at nodes.

unique and two subunique substitutions which differentiate *P. subfalcatum* from all other species of the genus. One of our specimens of *P. distichaceum* from the Kurils (OK 2431) was also studied for *trn*L–F only, as its ITS was too heterogeneous, though several characteristic substitutions in its sequence clearly indicate its identity with another Kuril specimen, OK 2475.

Interestingly, the latter Kuril specimen of *P. distichaceum* (OK 2475) was collected in the same area where the Kuril’s *P. subfalcatum* was found, though they were collected on slopes of different mountains, at different altitudes (500 and 150 m a.s.l., respectively), and apparently in places with different bedrocks. Furthermore, this specimen totally lacks propagules, and thus was included in our analysis to check its identity. This confirms the conclusion of Li *et al.* (2015) that the lack of propagules may occur in *P. distichaceum*, and the type specimen of this species also lacks them. Kuril specimen, OK 2475, is totally green, apparently because of the growth in deep shade (most Kuril specimens are at least partly cherry-red), and this might explain the underdevelopment of propagules.

In terms of biogeography, *P. subfalcatum* is one more addition to the Arcto-Tertiary relic group of species, with the disjunction between East Asia and eastern North America (Schofield & Crum, 1973; Schofield, 1988; Ignatov, 1993). Such species are more common in the easternmost part of Asia, including East China, Japan and Russian Far East, but some of them reach the Baikal Lake area, e.g. *Brothera leana*, *Buxbaumia minakatae*, *Anomo don minor*, and *Pylaisiadelpha tenuirostris*.

Although we did not see Japanese specimen (QZJP85), the GenBank accession HQ665374 rather clearly indicates that *P. subfalcatum* occurs in Japan, although the species is probably not common there (a number of collections studied in MW, MHA, LE and H did not reveal it). In the Kuril Islands *P. subfalcatum* was collected only once, whereas *P. distichaceum* is more common.
Fig. 2. *Pseudotaxiphyllum subfalcatum* (from: Russia, Buryatia, 9.VIII.2011 Kazanovsky s.n., LE, MHA). 1 – habit, dry; 2 shoot apex with axillary propagules; 3 – upper laminal cells; 4 – median laminal cells; 5–7 – leaves; 8–10 – axillary propagula; 11 – basal laminal cells. Scale bars: 2 mm for 1; 1 mm for 5–7; 0.5 mm for 2; 200 μm for 8–10; 100 μm for 3–4, 11.

**Taxonomy**

**Key to the Identification of *Pseudotaxiphyllum* Species in Russia**

1. Leaves ±symmetrical, from ovate base abruptly or gradually narrowed into filiform acumen; axillary brood branches with leaflets along their bodies ……………………... **P. elegans**
   — Leaves asymmetrical, cultriform, acute at apex; axillary brood branches with leaflets restricted to their apices ……………………………………………………… 2

2. Axillary brood branches born in leaf axes throughout the stem, more than 3 cells wide …………………… **P. distichaceum**
   — Axillary brood branches born in leaf axes only at shoot apex, 2–3 cells wide …………………… **P. subfalcatum**


Plants small to medium-sized, light green, glossy, forming flat mats. Stems simple or irregularly branched, complanate-foliate. Leaves 1.1–2.0 mm long and ca. 0.5
Fig. 3. 1–4, 13: *Pseudotaxiphyllum elegans* (from: Russia, Chukotka, Lavrentia settlement, 10.VIII.1969 Afonina s.n., LE); 5–8, 14: *P. distichaceum* (from: Russia, Kunashir Island, Ignatov 06-1697, MHA); 9–12, 15: *P. subfalcatum* (from: Russia, Buryatia, 9.VIII.2011 Kazanovsky s.n., LE, MHA). 1–2, 5–6, 9–10 – axillary propagula; 3–4, 7–8, 11–12 – leaves; 13–15 – habit, dry. Scale bars: 2 mm for 13–15; 1 mm for 3–4, 7–8, 11–12; 200 μm for 1–2, 5–6, 9–10.
mm wide, spreading, often undulate, oblong to oblong-lanceolate, asymmetrical, cultriforme, acute; margins plane or narrowly recurved at base, serrate at apex, below serrulate to mid-leaf; costa short and double; mid-leaf cells linear-flexuose, 50–75(–88)×7–8 µm, at apex shorter, rhomboidal; alar cells not differentiated. Specialized asexual reproduction by axillary propagules (brood branches) usually present in leaf axils at shoot tips; brood branches numerous, 0.2–0.6 mm long, twisted-verniform, 2–3 cells wide, with 1–5 acute, erect leaflets at apex. Gametangia not seen in specimen from Russia. [Dioicus. Sporophytes unknown.]

Specimens examined: RUSSIA: Republic of Buryatia, Kabansky Distr., Baikalsky Nature Reserve, right bank of Ydriyinyaya River, 51°25’38.1”N, 104°54’21.0”E, 565 m alt., SW-faced slope ca. 20° with rock outcrops, mixed forest of birch, Siberian pine, spruce and fir with Bergenia crassifolia, 9.VIII.2011, Kazanovsky s.n. (LE, MHA). The plant was presumably collected on ledge of rock outcrop. Sakhalinskaya Province, South Kuril Islands, Iturup Island, Medvezh’ya Bay, 45.416°N, 148.826°E, 150 m alt., creek in the lower part of N-faced slope of Medvezh’ya Mt., 15.IX.2015 Pisarenko op05831 [MW 9060912, duplicate from NSK].

Distribution and ecology. Until now, P. subfalcatum was known in eastern North America, as well as Central and South America; in N. America it occurs from Arkansas and Georgia to Ontario and Quebec, typically growing on moist cliffs of acid rocks, on ledges, and in crevices, sometimes on peaty soil in woods (Crump & Anderson, 1981; Ireland, 2014). In Asiatic Russia it was also collected in shaded and rather moist environments, especially in Iturup Island, where this species grows on volcanic rocks along small stream covered with soil and moist debris. This creek runs on slope of old volcano covered by secondary vegetation formed after fire by Sasa sp. thickets and sparse trees of Larix kantschatica; these thickets are so dense, that form a pipe-like corridor 2–4 m wide along the stream bed and making this space wet and shady.

Differentiation. Specimens of P. subfalcatum from Buryatia are similar to the widespread SE Asia P. distichaceum, in their asymmetrical, cultriform leaves with inaperturate leaflets of the brood branch-axes; but the leaf cells are larger and narrower, 2–3 cells wide, whereas in P. distichaceum they are longer and wider, 4–5 cells wide (Fig. 3).

Acknowledgements

We are grateful to Bruce Allen and John Atwood for providing information on the morphology of the American specimens of Pseudotaxiphyllum subfalcatum. The contribution of O. M. Afonina was carried out within the framework of the institutional research project “Flora of lichens and bryophytes of Russia and phytogeographically important regions” (AAAA-A19-119020690077-4). Molecular studies of A. V. Fedorova and E. A. Ignatova were supported by RSF18-14-00121. The contributions of S. G. Kazanovsky was supported by RFBR 18-04-00822.

Literature Cited


Appendix 1. Newly generated sequences [ITS / trnL-F]

Pseudotaxiphyllum distichaceum: Kuril Islands, Kunashir, Ignatov 06-1697 (MHA 9046680) isolate OK2431 [MW 8494242]; Kuril Islands, Iturup, Fedosov 15-2-183 (MW 9074998) isolate OK2475 [MN847623 / MN849423].

Pseudotaxiphyllum subfalcatum: Kuril Islands, Iturup, Pisarenko op05831 (MW 9060912), isolate OK2474 [MN847622 / MN849422]; Iturup Island, 9 Aug 2011 Kazanovsky s.n. (MHA), isolate OK2417 [MN847621 / MN849420].