CYRTO-HYPNUM VERSICOLOR (THUIDIACEAE, BRYOPHYTA) IN RUSSIA

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Abstract

Cyrto-hypnum versicolor is found for the first time in the Kuril Islands. This species was hitherto known in Russia only from the mainland, in Primorsky Territory. The species is extremely variable, and two collections from Russia represent opposite limits of its variation. The Kurilian specimen represents a marginal phenotype that is impossible to identify using keys in any floras. The illustrations and discussion on this variation is provided. The partial molecular phylogenetic analyses indicate a close relationship of Kurilian specimen with C. versicolor, although numerous differences in ITS sequences point out a necessity of a much broader analysis, making also the species promising for phylogeographic studies.

INTRODUCTION

The Far Eastern region is contrastingly different in its moss flora from the most part of Northern Asia. There are many temperate and subtropical relics in the area (Lazarenko, 1944; Bardunov & Cherdantseva, 1982; Ignatov, 1993).

Among other, the Far Eastern flora, as well as the whole East Asian one, is characterized by more numerous species of Thuidiaceae and Leskeaceae than any other longitudinal sector of the Holarctic (Bardunov & Cherdantseva, 1982). Despite of a careful exploration of Thuidiaceae in Japan and adjacent areas by Watanabe (1972), and recent revisions for China (Wu et al., 2002) and Japan (Noguchi, 1991), some plants from Russian Far East do not fully correspond descriptions, and some of them there described as new taxa. Among them, Thuidium thermophilum Czernyadjeva from thermal fields of Kamchatka (Czernyadjeva et al., 2006), and the Actinothuidium hookeri subsp. boreale Ignatov, Ignatova, Z. Iwats. & B.C. Tan, a northern segregate of a widespread East Asian species (Ignatov et al., 1999).

In the course of exploration of the termal habitats and their flora in Kuril Islands, one inegmatic specimen was collected by the first author. Plant grew on rock, with creeping stem, firmly attached to substrate by rhizoids and represented in collection mostly by branches and branchlets, which surface cells were mammillose. Comparison with the specimens known in Russia, as well as with the keys and descriptions in various Asian floras and taxonomic revisions of Thuidiaceae did not allow its certain identification.

A molecular phylogenetic analysis of the DNA sequence data was recently developed as a powerful method for resolving problems of difficult species delimitation, confirming or rejecting separate status of a putatively new species, correcting generic and familial placement and stopping sometimes endless debates on these mat-

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The specimen in question was sequenced for ITS and trnL-F, the most common markers available in GenBank for mosses. We used an alignment prepared for publication of Ignatov et al. (2007), which resulted in a tree principally similar to other analyses of the group (e.g., Huttenen et al., 2012).

Bayesian analyses were implemented in MrBayes v3.1.2 (Huelsenbeck & Ronquist, 2001), with four compartments: trnL-F, 5.8 rDNA, ITS1, ITS2. The HKY+I model was used for the two former regions, while the internal spacers were analysed with GRT+I model, according to model test (Lanfear et al., 2012). Three parallel runs were implemented, each with five chains and 1000000 generations (25% burnin), a temp parameter runs were implemented, each with five chains and 1000000 generations (25% burnin), a temp parameter

**RESULTS**

Preliminary tests with the separate chloroplastic and nuclear partitions showed that the phylogenetic signal from the chloroplast trnL-F is low and the tree based on this marker is poorly resolved. At the same time, the analyses of ITS and ITS+trnL-F gave subidentical topologies, differing only in the position of our Kurilian specimen in question. In both cases it was found within a clade of *Cyto-hypnum*. The difference was that in the ITS+trnL-F tree the Kurilian plant was found in paraphyletic grade to the crown formed by *C. minutulum*, and near Chinese specimen of *C. versicolor* (GenBank sequence, ITS only available). In the analysis based on ITS only, Kurilian plant and Chinese *C. versicolor* were resolved in a clade (PP=1) nested in polytomy of *C. minutulum*. As the difference in results apparently related to the absence of trnL-F data, only ITS is shown here (Fig. 1).

The obtained tree provides a topology in general corresponding to the natural units found in Hypnales by Huttenen et al. (2012). Among them the high support (PP=0.99) was found for the clade of Amblystegiaceae +Lindbergiaceae +Pseudoleskeellaceae +Leskeaceae +Thuidiaceae. Two latter families, the Thuidiaceae and Leskeaceae, formed a clade with the maximal support. The taxa of this clade formed a broad polytomy with several smaller clades and individual species, which points out the difficulty in the segregation of Leskeaceae from Thuidiaceae, discussed already by Gardiner et al. (2005) and Ignatov et al. (2007).

However, the clade formed by *Thuidium* and *Cyto-hypnum* obtained a maximal support in this analysis. Moreover, all *Thuidium* and all *Cyto-hypnum* species within this clade were also resolved as monophyletic and with the maximal support.

An enigmatic moss from Kuril Islands appeared deeply nested in *Cyto-hypnum*, in a subclade with the Chinese specimen of *C. versicolor* (PP=1.0), and this subclade was sister to *C. minutulum* subclade. Thus, all further discussion will be about this genus.

**DISCUSSION**

There were two problems with identification of this difficult Kurilian plant. It differed from the descriptions of *Cyto-hypnum versicolor* in the following points: paraphyllia were absent on stem; truncate paraphyllia-like structures with multipapillose apical cell occurred only around branch primordia; all surface cells of stem, branches and branchlets were mammillosse, with mammillae rather even in shape and size throughout branches and branchlets and stem fragments from its middle part (cf. Fig. 1: 10), as the uppermost parts of stem were missing in the collection.

The second problem was in a contrasting difference in morphological characters between Kurilian plants and the only previously known collection of *C. versicolor* in Russia, from Primorsky Territory. Primorsky' specimen had larger size of plants as compared to Kurilian one, dense paraphyllia on stem, paraphyllia totally absent on branches and branchlets, and branch leaves long acuminate, with multipapilllose apical cell.

If these two specimens were compared alone, their conspecificity would be definitely rejected.

At the same time, a cross-comparison of the descriptions provided by Watanabe (1972), Norris & Koponen (1985), Noguchi (1991), Touw (2001), Fang & Koponen (2001), and Wu et al. (2002) convinced us that this is not impossible. It seems that the range of variation of *C. versicolor* was never fully described, but it appears from the superimposing facts of variation from the comments made by different authors, which are as follow:

- “Paraphyllia on stem, rare on branches, absent on branchlets” (Fang & Koponen, 2001, page 31).
- “Paraphyllia abundant on stems, sparse on branches and lacking on secondary branches, usually short, 2–4 cells long, rarely foliose, ending in a truncate or acute, papillose cell, simple on stem, rare on branches, absent on branchlets” (Noguchi, 1991, page 862).
- “Paraphyllia densely growing on stems, usually simple, 2–6 cells long, apical cells truncate and papillose, rarely acute and branched” (Wu et al., 2002, page 177).
- “Paraphyllia crowded on main stem, rarely also on branches, but fewer on the latter, filiform, 1–3 cells long, apical cells crowned with 2–4 papillae (Watanabe, 1972, page 182).
- “Stem paraphyllia to 5 cells long, scattered to closely set; intermediate cells smooth to strongly papillose; api-
Fig. 1. Bayesian phylogenetic tree of Leskeaceae and Thuidiaceae s.ampl. based on ITS sequences. Posterior probabilities >0.7 are shown at branches.
**Fig. 2.** *Cyrto-hypnum versicolor* (Hornsch. ex Müll. Hal.) W.R. Buck & H.A. Crum (from: Russia, Kunashir Island, Koroteeva 15-3/2-7, MHA). 1 – habit, dry; 2–3 – capsules; 4 – stem transverse section; 5 – primary branch transverse section; 6, 10 – habit, wet; 7 – part of primary branch with paraphyllia; 8 – cells of distal portion of branch leaf from primary branch; 9 – cells of distal portion of branch leaf from secondary branch; 11 – surface cells of stem; 12 – basal cells of primary branch leaf; 13 – primary branch leaf transverse section; 14–16 – primary branch leaves; 17–18 – stem leaves; 19 – pearichaetial leaf; 20 – part of peristome; 21–22 – secondary branch leaves. Scale bars: 5 mm for 1; 2 mm for 2–3, 6; 1 mm for 10; 0.5 mm for 19; 0.2 mm for 14–18, 21–22; 50 μm for 20; 100 μm for 4–13.
cal cells in most longer paraphyllia truncate and pluripapillose, occasionally acute and smooth or weakly papillose (in the smaller paraphyllia more often so); branch paraphyllia few to numerous, often unicellular and mammilliform; branchlets naked or with mammilliform paraphyllia” (Touw, 2001, page 76). Commenting variation, Touw (2001, page 79) added: “The branches are strongly paraphyllose in East Asia, whereas they are often naked or nearly so in tropical Asia and Hawaii. The paraphyllia diminish in size towards the branch tips, and acute ones are most frequent there. Ultimately, they often consist of a single mammilliform cell”.

Thus, even the most detailed description of Touw says nothing about mammillose cortical cells, only about mammilliform unicellular paraphyllia.

There is also an inconsistency in spore size: they were reported as 10–17 μm (Wu et al., 2002), 7–15 μm (Touw, 2001), 7–12 μm (Fang & Koponen, 2001), or 16 μm (Watanabe, 1972).

Another discrepancy has been already noted by Fang & Koponen (2001): seta surface was described as papillose by Robinson (1968) and Gangulee (1978), but as smooth by Watanabe (1972) and Norris & Koponen (1985); the latter being correct, according to Fang & Koponen (2001).

The polymorphism of the species resulted in a numerous superfluously described species: Touw (2001) listed as many as 15. The most fascinating example is pro-
Fig. 4. *Cyrtol-hypnum versicolor* (Kuril Islands), showing mammillae on branches (3–6) and branchlets (1–2), and mammillose branch and branchlet leaves, except almost smooth-celled most proximal branch leaves (5).
Fig. 5. *Cryo-hypnum versicolor* (1, 3, 5: from Primorsky Territory; 2, 4, 6–7: from Fukushima area in Honshu, MHA), showing mammillae on stem (2, 5), branches (4), branchlets (6), and leaves (2–4, 6).
vided by Fang & Koponen (2001), about Bescherelle who described four species new to science from two collections, and three of them appeared to be synonyms of *C. versicolor*.

Thus, our intension to describe an unknown Kurilian plant as a new species was stopped. At the same time, having two so dissimilar populations, and basing on only two specimens, we faced the difficulty with providing the description of *C. versicolor* for the moss flora of Russia, if based strictly on the Russian collections. We found no better solution than to separate characters of plants from two populations, showing states of some characters separately [in brackets, for the plants from Primorsky Territory] and {in braces, for the Kurilian plants}. This way of presentation can be useful in future, if additional morphological and/or molecular studies challenge the conspecificity of the mainland and Kurilian populations.

**TAXONOMY**

*Cyrto-hypnum versicolor* (Hornsch. ex Müll. Hal.) W.R. Buck & H.A. Crum

Figs. 2–5

Plants [small] or {minute}, green to light green, not glossy, forming a tiny patches on [rocks, soil and rotten wood] or [on rock surfaces]. Stem [ca. 15 mm long] or {ca. 0.5 mm long}, [loosely attached to the substrate] or {creeping on substrate, tightly attached by numerous rhizoids}, central strand absent, bipinnately branched; primary branches [to 4 mm long, moderately spaced, arranged on both sides of stem] or {2.5–3 mm long, densely arranged on one side of stem and arising vertically from it}; secondary branches {1.0–1.2 mm long} or {0.5–0.8 mm long}; surface of stem and branches {smooth} or {mammillose}; {stem covered with dense paraphyllia on primary branches paraphyllia sparse or absent} or {paraphyllia sparse on stem and primary branches}, paraphyllia small, 40–45 μm long, narrow and truncate at apex or foliose. Stem leaves [regularly arranged] or {sparsely due to dense branching pattern}, {ca. 0.5 mm long and 0.3 mm wide} or {ca. 0.25 mm long and 0.15 mm wide}, triangular, with narrow acumen filled by costa; costa strong, gradually narrowing distally, excurrent. Primary branch leaves {0.3–0.35 mm long and 0.15 mm wide} or {0.21–0.27 mm long and 0.09–0.1 mm wide}, ovate-lanceolate, [acuminate, with obtuse apical cell] or [shortly acute or subobtuse at apex, with acute or obtuse apical cell], shallowly keeled above; margins plane, densely serrate; costa single, gradually narrowing distally, ending well below apex, smooth, looking pale in contrast with dim lamina areolation; lamina cells uniform within the whole leaf, subquadrate and short rectangular, 5–8 μm long and 4–6 μm wide, moderately thick-walled, with single high papilla on dorsal leaf surface or, at places, on both surfaces. Secondary branch leaves ovate, 0.1–0.18 mm long, 0.05–0.06 mm wide, {acute} or {rounded at apex}, with plane margins, serrate, lamina areolation like in primary branch leaves. Autioous, sporophytes {not seen} or {numerous}. {Perichaetia large, whitish, perichaetal leaves ca. 2.3 mm long, with oblong base and long triangular apex, not plicate, margins plane, finely serrulate at places, costa strong, narrowing distally, excurrent. Seta ca. 1 cm long, reddish-brown, smooth, straight or slightly bent shortly below the capsule. Capsules short, slightly curved, with ovate urn and short neck, urn ca. 0.9 mm long and 0.5 mm, operculum conic, with oblique beak ca. 0.55 mm long, annulus revoluble, consisting of two rows of inflated cells. Spores 7–9(–11) μm, smooth. Calyptra not seen).


Fang & Koponen (2001) confirmed this species in China only from the southern areas, expressing doubts for some recored from the north of the country. The locality in Primorsky Territory of Russia makes the occurrence of the species in northern China rather likely.

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**LITERATURE CITED**


IVANOVA, E.I., N.E. BELL, O.I. KUZNETSOV & O.V. IVANOV. 2010. The genus Actinothuidium (Hookeriaceae, Bryophyta) in Russia: combined genetic analyses. – ITS // trnL-F are given, or if ITS1 and ITS2 have different accession numbers, then format is ITS1_ITS2 // trnL-F.

Appendix 1. Specimen data for newly obtained sequences and GenBank accession numbers for all sequences used in the analyses. ITS // trnL-F are given, or if ITS1 and ITS2 have different accession numbers, then format is ITS1_ITS2 // trnL-F.


**Previously used and GenBank sequences:** Abietinella abietina AY009802 // AY009850; Abietinella abietina AJ417494 // –; Actinothuidium hookeri AY568547 // –; Amblyphystegium serpens AF168152 // AY009827; Anacampstodon splachnoides AY009810 // AY009816; Anomodon rugulos A1277232_A1288420 // AF161116; Bologaya mittenii FM161080 // AM990347; Claoipodium rostratus AY528899 // AY528896; Claoipodium crispifolium AY695739 // AY695778; AY683583; Cyto-hypnum versicolor AY568545; Haploecladium angustifolium AY528884_AY528885 // AY527129; Haploclymenium triste AY568551 // –; Haploclymenium triste FM161113 // –; Helodium blandowii AY009803 // AY009852; Heteroclymenium procurrens AY695782 // AY683578; Hookeria lucens A1252137 // AF215906; Lescuraea incurvata AY693661 // AY683595; Leskea polycarpa AY528889_A516151 // AY527134; Lindbergia brachyptera FM161151 // AY683571; Lindbergia grandiretis JF280965 // –; Miyabaea rotundifolia FM161155 // AM990412; Platycladiya jugernannioides AF168162 // AY009857; Pseudoleskeella catenulata AY695747 // AY683578; Pseudoleskeella serpentinense AY695748_AY695775 // AY683580; Pseudoleskeopsis zippelli FM161206 // –; Rauiella fujisana AY568546 // AY683600; Sasaoka aomoriensis JF280974 // JF280981; Thamnophyllum alopocoides FM161218 // –; Thuidium philiberti A146442 // –; Thuidium cymbifolium AY568542 // –; Thuidium kamedae Sakurai AY568541 // –; Thuidium glaucinoides AY568544 // –; Thuidium pristocalyx AY568540 // –; Thuidium tamariscinum AF023770 // –; Thuidium thermophilum EF368013 // EF368012.