Abstract

It has been controversial whether Betula tatewakiana, a dwarf birch distributed in Hokkaido of northern Japan, is an endemic species or a synonym of B. ovalifolia broadly distributed in northeast Asia. The endemic hypothesis is based on the idea that B. tatewakiana is diploid while B. ovalifolia is tetraploid and that they are separated based on the ploidy level; however no chromosome data have actually been published before. Resolving the taxonomic problem is crucial also in judging the conservation priority of B. tatewakiana in a global perspective. Our chromosome observation revealed that B. tatewakiana is tetraploid as well as B. ovalifolia. Collaterally, we conducted morphological observation and clarified that B. tatewakiana is morphologically identical to B. ovalifolia in white hairs and dense resinous glands respectively on adaxial and abaxial leaf surfaces, based on which they are different from closely related species in the same section Fruticosae. We concluded that the hypothesis that B. tatewakiana is a Hokkaido endemic based on the ploidy level is not supported and that B. tatewakiana should be merged with B. ovalifolia.

Keywords

Betula, chromosome number, dwarf birch, endangered species, Hokkaido, Japan, polyploid, Russian Far East, synonymization, wetland
books and floras in Japan (Murata 1979, Ito 1981, Ohba 2006, Takahashi 2015), the idea to support *B. tatewakiana* was claimed again (Watanabe 1995) and the taxonomic problem still remains (Takahashi 2013, Takahashi et al. 2013, Nemoto 2016). Here, we tentatively used the name *B. tatewakiana* and later discuss its taxonomy and proper name based on our result.

The taxonomic problem of *B. tatewakiana* and *B. ovalifolia* stems from the confusion in their ploidy level. Watanabe (1995) claimed that *B. tatewakiana* is diploid while *B. ovalifolia* is tetraploid and he recognized *B. tatewakiana* as the Japanese endemic restricted to Hokkaido. This idea, however, was originally reported in a conference abstract without images of the chromosomes (Watanabe & Somego 1991) and has never been published, but repeatedly mentioned in following studies (Nemoto 2016). On the other hand, Nagamitsu (2004) did not separate the two species and treated *B. ovalifolia* from Hokkaido as a tetraploid species based on Provatoba (1995), which actually didn’t report the chromosome number of *B. ovalifolia* but of a hybrid between *B. ovalifolia* and *B. exilis*. A flow cytometric study of the genome size evolution in the genus *Betula* suggested that *B. ovalifolia* from the Asian continent is tetraploid (Wang et al. 2016), but no information based on chromosome observation exist about the ploidy level of *B. tatewakiana* and *B. ovalifolia*.

In this study, to resolve the taxonomic problem of *B. tatewakiana*, we conducted chromosome observation and determined the ploidy level. We collaterally conducted morphological observation of *B. tatewakiana*. Regarding *B. ovalifolia*, there are two closely related species in the same section *Fruticosae*, i.e., *B. humilis* Schrank (1789: 420) and *B. fruticosa* Pall. (1776: 758) and *B. ovalifolia* is distinguished from the two species by white hairs on adaxial leaf surface (vs. glabrous in *B. humilis* and *B. fruticosa*) and by densely resinous glands on abaxial leaf surface (vs. lack of glands in *B. humilis*) (Kuzeneva 1985, Li & Skvortsov 1999). In previous studies which did not accept *B. tatewakiana*, these traits have not been well compared between *B. tatewakiana* and *B. ovalifolia*. Resolving the taxonomic problem and assess the endemic status of *B. tatewakiana* would also help planning its conservation. *Betula tatewakiana* is distributed only in two localities in Japan, i.e., Sarabetsu and Nishibetsu mires in eastern Hokkaido (Fig. 1-B). As a result of the exploitation of the mires, remaining habitats are only 3 and 16 ha in Sarabetsu and Nishibetsu mires, respectively (Takahashi 2013). Open ditches excavated inside and outside the mires are increasingly drying the habitats of *B. tatewakiana* and thereby it is red-listed at national and prefectural levels (Hokkaido 2001, Ministry of the Environment of Japan 2018). Whether it is endemic or not is related to its conservation priority in a global perspective; on the other hand, if it is the same species as *B. ovalifolia* broadly distributed in northeast Asia, effective conservation should be planned considering genetic connectivity with conspecific populations abroad. This study is expected to provide basic information essential for the conservation of the species.

**Material & Methods**

**Determination of ploidy level**

We collected seeds of *B. tatewakiana* from six and five individuals from Sarabetsu and Nishibetsu mires in Hokkaido, Japan; seeds of *B. ovalifolia* were collected from one individual in Sikhote-Alin Nature Reserve in Primorsky Krai, Russian Far East (Table 1). Collected seeds were dried with silica gel and stored at 4°C. Seeds were sowed on vermiculite and germinated at 25°C day / 8°C night condition for two weeks. After germination, root tips were collected and pretreated by 0.002 M 8-hydroxyquinoline solution for 24 hours at 4°C in dark condition. Next, the root tips were fixed by Farmer’s solution (glacial acetic acid : 99 % ethanol = 1 : 3) at 4°C in dark condition. After fixation, the root tips were macerated in 1 N HCl for 18 minutes and stained with 1 % aceto-orcein for 5 minutes and squashed on a slide. Metaphase chromosomes were observed using an optical microscope Zeiss Axio Imager A1 (Carl Zeiss, Jena, Germany), and pictured by Anyty 3R-DKMC01 (3R solution corp., Fukuda, Japan).
Morphological observations

To elucidate whether *B. tatewakiana* is morphologically identical to *B. ovalifolia* or not, we observed the key traits in the section *Fruticosae*: white hairs and dense resinous glands respectively on adaxial and abaxial leaf surfaces. For *B. tatewakiana*, specimens examined were the holotype of *B. tatewakiana* (H. Suzuki & M. Ohki, s.n. with handwriting “Type”) in the herbarium of Hokkaido University Museum (SAPS) and our collections of 51 and 45 plants from Sarabetsu and Nishibetsu mires, that were deposited in the herbarium of Hokkaido University Botanic Garden (SAPT) (Appendix 1). For *B. ovalifolia*, our collections of 38 specimens from Primorsky Krai in Russia Far East were used (SAPT, Appendix 1).

Results

Ploidy level

Somatic chromosomes at metaphase were approximately 1.0 µm long in both *B. tatewakiana* (Fig. 2 A–D) and *B. ovalifolia* (Fig. 2 E, F). The centromere positions could not be determined because of the small sizes of the chromosomes. The result of chromosome counts is summarized in Table 1. In *B. tatewakiana* from Sarabetsu mire, 3 individuals had 56 chromosomes (HUBG 14746 A, 14746 E, and 14746 H), 2 individuals had ca. 52 chromosomes (HUBG 14746 B, D) and 1 individual had ca. 50 chromosomes (HUBG 14746 F). In *B. tatewakiana* from Nishibetsu mire, 4 individuals had 56 chromosomes (Yuki Shiotani 1, 26, 29, 30) and 1 individual had ca. 53 chromosomes (Yuki Shiotani 27). In *B. ovalifolia* from Primorsky Krai, 1 individual had 56 chromosomes (Koh Nakamura 14198).

Morphological traits

The holotype of *B. tatewakiana* had white hairs and dense resinous glands respectively on adaxial and abaxial leaf surface (Fig. 3 A, B). Our collections of *B. tatewakiana* also had white hairs and dense resinous glands on adaxial and abaxial leaf surface, respectively (Fig. 3 C, D) and no morphological difference was recognized between the samples from Sarabetsu and Nishibetsu mires. In *B. ovalifolia*, our collections from Primorsky Krai had white hairs and dense resinous glands respectively on adaxial and abaxial leaf surface as well as *B. tatewakiana* (Fig. 3 E, F).

Discussion

Merger of *B. tatewakiana* to *B. ovalifolia*

In our chromosome observation, the samples of *B. tatewakiana* from Sarabetsu and Nishibetsu mires had $2n = 50$ (one sample), 52 (two samples), 53 (one sample), and 56 (seven samples) chromosomes (Table 1). The chromosomes were too small (approximately 1.0 µm long) to observe clearly and the chromosome count variation may need further verification; however, it would be safe to say that *B. tatewakiana* is tetraploid because the basic chromosome number is 14 in the genus *Betula* (Erikkson & Jonsson 1986) and the diploid count should be $2n = 28$. Watanabe & Somego (1991) reported that *B. tatewakiana* is diploid, although no images of the chromosomes were presented. Thus, the possibility that there are both diploid and tetraploid in *B. tatewakiana* is not totally denied. However, his report was gametophytic count and according to the author Watanabe the chromosome image was unclear (personal communication). For this reason, *B. tatewakiana* is highly likely to be tetraploid. Our chromosome count of *B. ovalifolia* was $2n = 56$. This is consistent with the flow cytometric study that suggested that *B. ovalifolia* from the Asian continent is tetraploid (Wang et al. 2016). Therefore, the idea to separate *B. tatewakiana* from *B. ovalifolia* based on the ploidy level (Watanabe & Somego 1991, Watanabe 1995) is not supported because both species are tetraploid. Hence, *B. tatewakiana* should be merged to *B. ovalifolia*. The observation of the morphological traits also supports the merger of *B. tatewakiana* to *B. ovalifolia*. The two species are morphologically identical in white hairs and dense resinous glands respectively on adaxial and abaxial leaf surfaces, based on which they are different from closely related dwarf birch species in the same section *Fruticosae*. 
Implications for conservation

Betula tatewakiana is recognized as a synonym of *B. ovalifolia* as discussed above, and thereby it is not a Japanese endemic species. Hereafter the Hokkaido populations are called *B. ovalifolia*. Because *B. ovalifolia* is broadly distributed in northeast Asia, i.e., Russian Far East, northeast China, north Korea, and northern Japan, the conservation priority of the species may not be high in a global perspective. On the other hand, the Hokkaido populations represent only island populations disjunct from continental populations. The species had likely moved southward during glacial periods and retreated northward in warmer periods, and the Hokkaido populations are considered to be relict populations (Takahashi 2013). The Hokkaido populations can be reproductively isolated from the continental populations and can have a unique gene pool that deserves conservation. Also, domestically in Japan, *B. ovalifolia* is distributed only in Sarabetsu and Nishibetsu mires in Japan and deserves conservation as national resource. If there exists geneflow among Hokkaido and continental populations, effective conservation should be planned considering genetic connectivity with populations abroad. Population genetics of *B. ovalifolia* in northeast Asia for conservation is the topic of our future investigation.

Acknowledgement

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References


Appendix 1. Specimens for morphological observation.

**Betula tatewakiana**

Japan, Hokkaido: Sarabetsu village, 18 August 1958, (H. Suzuki & M. Ohki, s.n. with handwriting “Type”, SAPS); Sarabetsu mire, 5 June 2017, Yuki Shiotani 65–115 (51 specimens, SAPT); Nishibetsu mire, 7 June 2017, Yuki Shiotani 1–26, 31–49 (45 specimens, SAPT)

**B. ovalifolia**

Russia, Primorsky Krai: Terney, 22 July 2016, Koh Nakamura 14169–14195, 14197, 14198 (29 specimens, SAPT); Terney, 23 July 2016, Koh Nakamura 14289–14297 (9 specimens, SAPT)
Figure legends

FIGURE 1. Species distribution ranges of *Betula ovalifolia* (A) and *B. tatewakiana* (B).

FIGURE 2. Somatic chromosomes at metaphase of *B. tatewakiana* and *B. ovalifolia*. Photomicrographs of *B. tatewakiana* from Sarabetsu mire (*A*, 2*n* = 56: HUBG 14746) and Nishibetsu mire (*C*, 2*n* = 56: Yuki Shiotani 29), and *B. ovalifolia* from Primorsky Krai (*E*, 2*n* = 56: Koh Nakamura 14198) are shown. *B*, *D*, and *F* are drawings of *A*, *C*, and *E*, respectively. Scale bar is 5 µm.

FIGURE 3. Leaf traits of *B. tatewakiana* and *B. ovalifolia*. White hairs on adaxial leaf surface (*A*, *C*, *E*) and densely resinous glands on abaxial leaf surface (*B*, *D*, *F*) are shown for the holotype of *B. tatewakiana* (*H*. Suzuki & M. Ohki, s.n., *A* & *B*), *B. tatewakiana* of our collection (*Yuki Shiotani 38, C & D*), and *B. ovalifolia* in Russia (*Koh Nakamura 14188, E & F*). Scale bar is 1 mm.
TABLE 1. Chromosome counts of *Betula tatewakiana* and *B. ovalifolia*.

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Sampling site</th>
<th>Chromosome counts</th>
<th>Voucher no.</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>B. tatewakiana</em></td>
<td>Sarabetsu mire, Sarabetsu village, Hokkaido, Japan</td>
<td>56</td>
<td>HUBG* 14746 A, E, H</td>
</tr>
<tr>
<td></td>
<td>Hokkaido, Japan</td>
<td>52</td>
<td>HUBG 14746 B, D</td>
</tr>
<tr>
<td></td>
<td>Nishibetsu mire, Betsukai town, Hokkaido, Japan</td>
<td>50</td>
<td>HUBG 14746 F</td>
</tr>
<tr>
<td></td>
<td></td>
<td>56</td>
<td>Yuki Shiotani 1, 26, 29, 30 (SAPT)</td>
</tr>
<tr>
<td><em>B. ovalifolia</em></td>
<td>Sikhote-Alin Nature Reserve, Terney, Primorsky Krai, Russia</td>
<td>56</td>
<td>Koh Nakamura 14198 (SAPT)</td>
</tr>
</tbody>
</table>

*HUBG: living collections of Hokkaido University Botanic Garden*