Chromosome numbers of eight taxa of Aconitum L. in Korea and their systematic significance (Ranunculaceae)

Kyong-Sook Chung¹, Bomi Nam, Myung Soon Park, Jeong Ae Eom², Byeong-Un Oh¹ and Gyu Young Chung*

School of Bioresource Science, Andong National University, Andong 760-749, Korea
¹Department of Biology, Chungbuk National University, Cheongju 361-763, Korea
²Korea Forest Seed & Variety Center, Cheongju 380-941, Korea

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ABSTRACT: Various aneuploidy and polyploidy have been reported in the genus Aconitum L. (ca. 300 species worldwide, Ranunculaceae), and there is a demonstrated association between major lineage diversification and polyploidy. This study reports chromosome counts of eight Aconitum from Korea, including the first counts for A. japonicum Thunb. subsp. napiforme (H. Lév. & Variot) Kadota (2n = 32) and A. longecassidatum Nakai (2n = 16). The study also includes chromosome numbers for two taxa on the Critically Endangered species list in Korea. Among Korean native species, chromosome numbers in Aconitum subgenus Aconitum range from 2n = 16 to 2n = 64 with diverse levels of polyploidy (2x, 4x, and 8x), whereas Aconitum subg. Lycocoturn exhibits only diploids (2n = 16). Greater chromosome number diversity in subg. Aconitum than subg. Lycocoturn might explain higher species diversity within the former subgenus (more than 250 species worldwide). Investigating chromosome number diversity of Aconitum in a phylogenetic framework will be a critical step to understand species richness of the genus.

Keywords: Aconitum, Chromosome number, polyploidy, Ranunculaceae

Aconitum L. (Ranunculaceae) is a herbaceous perennial or pseudo-annual species characterized by hood-like, spurred, and clawed zygomorphic flowers with 2-petal (Kadota, 1987; Tamura, 1990; Li and Kadota, 2001). The genus consists of about 400 species worldwide, occurring mainly in temperate regions in the Northern hemisphere (circumboreal floristic region) with a high species diversity in Asia, southward into northern Mexico and Africa (Tamura, 1990; Li and Kadota, 2001; Park, 2007). Three subgenera, Aconitum subg. Aconitum, Aconitum subg. Lycocoturn (DC.) Peterrm., and Aconitum subg. Gymnacoturn (Stapf) Rapaiac, have been well accepted (Tamura, 1990; Li and Kadota, 2001; Lim and Park, 2001). Subg. Gymnacoturn (Stapf) Rapaiac has only one species, A. gymnandrum, endemic to China; and subg. Aconitum exhibits the greatest diversity in the genus with more than 250 species (Tamura, 1990; Li and Kadota, 2001). In Korea, twelve taxa in subg. Aconitum and seven taxa in subg. Lycocoturn occur, which are classified by root and stem morphology: Subg. Aconitum has tuberous roots without rhizomes (pseudo-annual, with caudices), whereas subg. Lycocoturn is characterized by fibrous roots with well developed rhizomes (perennial) (Li and Kadota, 2001; Park, 2007). Among 19 taxa, five species, A. austrocoreense Koidz., A. chishanense Nakai, A. pseudolaeve Nakai, A. pteropus Nakai, and A. quelpoense Nakai, are restricted to the Korean peninsula, and A. barbatum Pers. var. hispidum (DC.) Ser. and A. coreanum (H. Lév.) Rapaic have been listed as Critically Endangered species in Korea (Lee, 2008).

Along with high species diversity and various distribution patterns (i.e., cosmopolitan vs. endemcity; Li and Kadota, 2001), the genus exhibits great morphological diversity, which has resulted in a complex taxonomy (Brink, 1982; Kadota, 1987; Kosuge and Tamura, 1988; Ucelli et al., 2000; Miika et al., 2007; Yuan and Yang, 2008). In Korea, the Aconitum jalense Kom. complex with seven taxa and A. albiovalseum complex with four taxa have been a challenge to circumscribe infra-specific taxa for, and both chemical (flavonoid) and molecular (AFLP and DNA sequencing) evidence suggest strong hybridization and introgression might have occurred in the complex (Suh et al., 1997; Oh and Park, 1998; Lim et al., 1999; Lim and Park, 2001; Lee and Park, 2007). Within the complex,
Park and Oh (1997) distinguished A. chisanense from A. jalense subsp. jalense by chromosome numbers (2n = 16 vs. 2n = 32) in combination with morphological features such as the size and shape of leaves and pedicell hairs. Although cytogenetic characters have been very useful to understand circumscription and evolution of taxa (Stebbins, 1971; Kim, 1992; Guerra, 2008), only a few studies have been conducted for entire taxonomic groups in Korea (Sun et al., 1988; Lee et al., 2010; Chang and Chung, 2011).

Basic chromosome number of Aconitum is x = 8 with rare appearances of x = 9, 10, 13, and diverse ploidy levels from 2x to 8x (except for 7x) including aneuploidy (Schafer and La Cour, 1934; Gregory, 1941; Krasnikova et al., 1983; Krasnikov and Schaulo, 1986; Kadota, 1987; Friesen, 1991; Sha et al., 1995; Simon et al., 1999; Tomasz and Mitka, 2009). Polyploids and aneuploids in Aconitum have been reported, and chromosome number variation has been an important feature for understanding infragenetic classification and delimiting species boundaries (Schafer and La Cour, 1934; Yuan and Yang, 2006). Schafer and La Cour (1934) divided the genus into three sections based on haploid chromosome numbers: Lycocotonum with n = 8, Aithora with n = 16, and Eucratonum with n = 8, 12, 16, 24, 32, and 48. Although outstanding floral morphology makes Aconitum easy to distinguish from other genera in Ranunculaceae, broad variance of floral features has resulted in complex taxonomy. Chromosome numbers have been useful to delimit taxa and understand phylogenetic relationships in the genus (Park and Oh, 1997; Yuan and Yang, 2006). In Korean Aconitum species, diploids and tetraploids have been reported (Yang et al., 1993; Park and Oh, 1997). Lee (1967) reported chromosome numbers of four aconites: A. coreanum Rapais (2n = 32; (= A. coreanum (H. Lév.) Rapais), A. ustianum (2n = 32; (= A. jalense var. jalense), A. ustianum var. albiflora (2n = 32) and A. pseudokavae Nakai (2n = 16) with other native plants. Recently NIBR (2011) found out that A. astrolakoseense Koidz., one of the endemic species in the genus, has chromosome number of 2n = 16. However, chromosome number variation across the entire genus in Korea has not been discussed.

In the present study, we investigated chromosome numbers of eight Korean native Aconitum taxa and combined these findings eight previous counts to understand cytogenetic characteristics of the genus and improve understanding of infragenetic classification and evolution.

**Materials and Methods**

Eight taxa in Aconitum collected from various sites in Korea from June, 2010 to September, 2010 were cultivated in the greenhouse at the Andong National University (Andong, Gyeongsangbuk-do) and utilized for somatic chromosome number counting (Table 1). Actively growing root tips were cut and treated in triple distilled waters in 4°C for 24 hours, and then fixed in Carnoy’s solution (glacial acetic acid: absolute alcohol = 1 : 3). The roots were stored in a refrigerator for 24 hours prior to staining with Feulgen and 2% Aceto-carmine, and then the fixed roots were macerated using 1N HCl for 15 minutes. Stained materials were then squashed and made into

<table>
<thead>
<tr>
<th>Table 1. Materials and collection sites of the taxa examined. All voucher specimens are deposited at the Andong National University Herbarium (ANH).</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Taxon</strong></td>
</tr>
<tr>
<td>Aconitum subgenus Aconitum</td>
</tr>
<tr>
<td>A. ciliare DC.</td>
</tr>
<tr>
<td>A. coreanum (H. Lév.) Rapais</td>
</tr>
<tr>
<td>A. jalense Kom. subsp. jalense</td>
</tr>
<tr>
<td>A. japonicum Thunb. subsp. nipiforme (H. Lév. &amp; Vaniot) Kadota</td>
</tr>
<tr>
<td>Aconitum subgenus Lycocotonum (Staf.) Rapais</td>
</tr>
<tr>
<td>A. himalayense Pers. var. hispidum (DC.) Ser. (= A. sibiricum Poir.)</td>
</tr>
<tr>
<td>A. longicassidatum Nakai</td>
</tr>
<tr>
<td>A. pseudokavae Nakai*</td>
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</tbody>
</table>

*Endemic to Korea

Korean J. Pl. Taxon. Vol. 41, No. 3
Results and Discussion

1. Chromosome numbers of eight taxa of *Aconitum* L. in Korea

*Aconitum* L. subgenus *Aconitum*

1) *A. austrokoreense* Koidz. (Figs. 1-A, B)

This species is easily distinguished from other species by 3- or 5-palmately lobed leaves and white to light blue colored petals (Lee, 1980; Lee, 1996; Lee, 2002). The chromosome count found in this study are 2n = 16 and 18, which are conflict with the previous count of 2n = 16 by NIBR (2011). This species might have two cytotypes, but misinterpretation of squashed chromosomes is also possible. Clarification of the two counts is critical to understand evolution and endemicity of the species. This rare species has been only found in southern regions of the South Korea (Lee, 1980). The Korea Forest Service and the Ministry of Environment have put *A. austrokoreense* on the rare and/or protected species list (Lee, 2008). Understanding the genetic structure of the species at a population level is necessary to develop efficient conservation strategies (Ellstrand

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**Fig. 1.** Somatic chromosomes of eight taxa of genus *Aconitum*. A, B. *A. austrokoreense* Koidz. (2n = 16, 18); C. *A. ciliare* DC. (2n = 16); D. *A. coreanum* (J. L. Zv.) Rap. subsp. (2n = 32); E. *A. jaluense* Kom. subsp. *jaluense* (2n = 32); F. *A. japonicum* Thunb. subsp. *apifera* (H. L. Zv. & Vanin) Koidz. (2n = 32); G. *A. longecissidatum* Nakai (2n = 16); H. *A. pseudolaeve* Nakai; I. *A. barbatum* Pers. var. *hispidum* (DC.) Ser. (2n = 16).
and Elam, 1993).

2) *A. ciliare* DC. (Fig. 1-C)

*A. ciliare* is one of twin species and characterized by pedicels and sepals with abaxially retorse and appressed pubescences and glabrous ovaries (Lee, 1980; Li and Kadota, 2001). In China, the species is treated as a variety of *A. volubile* Palla ex Koehne, *A. volubile* var. *pubescens*, which is distinct from a variety with spreading pubescence on pedicels, sepals, and ovaries, *A. volubile* var. *volubile* (Li and Kadota, 2001). Counts from China and our own count from a Korean population agree on 2n = 16 (Yang et al., 1993; Li and Kadota, 2001), but a count from a Japanese population is 2n = 32 (Kadota, 1987). This conflicting result might reflect the taxonomic difficulty of the species associated with broad variation of morphological and geographic features. Taxonomic evaluation of the species complex should be conducted to determine whether the taxon is a variety of *A. volubile* as in Flora of China (Li and Kadota, 2001) or an independent species.

3) *A. coreanum* (H. Lév.) Rapais (Fig. 1-D)

Deeply parted leaves (segments dissected, ultimate lobes linear or linear-lanceolate) make the species distinct from other species (Lee, 1980; Lee, 2002). Our somatic chromosome number count of the species (2n = 34) is much greater than the previous count from a Chinese population (2n = 24 + 1B; Jin et al., 1998) but agrees with the count from a Korean population (Lee, 1967; A. coreanum Rapais). This might be caused by the unstable taxonomic status of the species in China (Li and Kadota, 2001). The species is listed as a rare, critically endangered species in Korea by the International Union for Conservation of Nature and Natural Resources (IUCN) (Lee, 2008). Clarification of the taxonomy of the target species is the first critical step to conserve this natural resource, since without this step it is impossible to utilize correct information for the target species. Examination of morphological features of the species covering its entire geographic distribution is desired to understand the species entity as a whole.

4) *A. jaluense* Kom. subsp. *jaluense* (Fig. 1-E)

This species is characterized by 3-parted leaves and is commonly found in forest margins (Lee, 1980; Lee, 2002), but populations with morphological, genetic features between *A. jaluense* Kom. subsp. *jaluense* and *A. japonicum* subsp. *napiforme* are found in southern parts of the Korean peninsula (Suh et al., 1997). Morphological characters of the individuals we used for chromosome squashes are not typical for the taxon but considered to be in a range of the morphological character variations discussed in Lim and Park (2001). Our own count of chromosome number is 2n = 32, the same as found in previous studies (Kadota, 1987; Park and Oh, 1997; Jin et al., 1998).

5) *A. japonicum* Thunb. subsp. *napiforme* (H. Lév. & Vaniot) Kadota (Fig. 1-F)

This taxon exhibits disjunct distribution in East Asia. It is found only in Jeju Island in Korea, Liaoning province in China, and several divisions in Japan (Lee, 1980; Li and Kadota, 2001; Park, 2007). Chromosome number counts from both China and Japan both indicate 2n = 32 (Kadota, 1987; Li and Kadota, 2001). This taxon has been included in the list of Least Concerned for extinction, and a survey of habitats and genetic information for the subspecies has been needed to develop conservation strategies (Lee, 2008). For the first time, this study reports a chromosome count of the taxon from a Korean population, which is congruent with the previous counts.

*Aconitum* subgenus *Lycocotonum* (Staf.) Rapais

6) *A. longecassidatum* Nakai (Fig. 1-G)

2n = 16 for the species is the first chromosome number count. This species has twining stems and 3-7 lobed leaves, and is morphologically very similar to *A. pseudolave*, one of the endemic species (Park, 2007). However, the latter has purple flowers, whereas the former has white ones (Lee, 1980; Lee, 2002).

7) *A. pseudolave* Nakai (Fig. 1-H)

This is one of the endemic species in Korea, found in all provinces. Our somatic chromosome number agrees with the previous count, 2n = 16 (Lee, 1967; *A. pseudolave* var. *erectum*). The species is characterized by small (ca. 2 cm long), purple flowers in branched racems (Lee, 1980; Lee, 2002).

8) *A. barbatum* Pers. var. *hispidum* (DC.) Ser. (= *A. sibiricum* Poir.) (Fig. 1-I)

This taxon is a Critically Endangered species, so it is urgent to understand natural populations and survey the genetic structure of the variety to develop conservation programs (Lee, 2008). Here, we provide chromosome number of 2n = 16 from a Korean population for the first time. Previous reports from several Chinese populations found the same chromosome number (Yang, 2001; Yuan and Yang, 2006).

2. Chromosome number variation in *Aconitum* L.: Polyploidy and aneuploidy

Chromosome numbers of *Aconitum* in Korea range from 2n = 16 to 2n = 64 with various cytotypes in four species: *A. austrokoreense*, *A. kusnezoffii*, *A. volubile*, and *A. umbrosum*.
<table>
<thead>
<tr>
<th>Taxon</th>
<th>Chromosome number (2n)</th>
<th>Reference</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Aconitum</em> subg. <em>Aconitum</em></td>
<td>16</td>
<td>Gurzenkov (1973); Yang (1996); Lee (2002)</td>
<td>NE China (Manchuria), Korea, Russia (Amur, Sakhalin, Ussuri)</td>
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<tr>
<td><em>A. austrokoreense</em> Koidz.*</td>
<td>16</td>
<td>NiBR (2011), Present study</td>
<td>Korea (Chungnyeong-san, Geumho-san, Yonggibong, GR; Jiri-san, GN; Baekun-san, JN)</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>Present study</td>
<td></td>
</tr>
<tr>
<td><em>A. chiisanense</em> Nakai*</td>
<td>16</td>
<td>Park and Oh (1997)</td>
<td>Korea (Seorak-san, GW; Jiri-san, GN)</td>
</tr>
<tr>
<td><em>A. ciliare</em> DC.</td>
<td>16</td>
<td>Yang et al. (1993); Li and Kadota (2001); Present study</td>
<td>NE China including Manchuria, Japan, Korea, Russia (Amur)</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Kadota (1987)</td>
<td></td>
</tr>
<tr>
<td><em>A. coreanum</em> (H. Lév.) Rapaics</td>
<td>24+1B</td>
<td>Jin et al. (1998)</td>
<td>N China including Manchuria, Korea, Mongolia, Russia</td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Lee (1967; A. coreanum Rapaics), Present study</td>
<td></td>
</tr>
<tr>
<td><em>A. jahense</em> Kom. subsp. <em>jahense</em></td>
<td>32</td>
<td>Kadota (1987); Park and Oh (1997); Jin et al. (1998); Present study</td>
<td>N China including Manchuria, Japan, Korea, Russia (Ussuri)</td>
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<tr>
<td><em>A. japonicum</em> Thurb. subsp. <em>napiiforme</em> (H. Lév. &amp; Vanini) Kadota</td>
<td>32</td>
<td>Li and Kadota (2001), Present study</td>
<td>NE China (Manchuria), Japan, Korea</td>
</tr>
<tr>
<td><em>A. kaszezoffii</em> Rehb.</td>
<td>16</td>
<td>Yang et al. (1993)</td>
<td>N China including Manchuria, Korea, Russia (Amur, Ussuri)</td>
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<td></td>
<td>24</td>
<td>Jin et al. (1998)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>32</td>
<td>Shang and Lee (1984); Sha et al. (1995); Yang (1996); Kadota (1987)</td>
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<td></td>
<td>32+1B</td>
<td>Jin et al. (1998)</td>
<td></td>
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<tr>
<td><em>A. monanthum</em> Nakai</td>
<td>Unknown</td>
<td></td>
<td>NE China (Manchuria), Korea</td>
</tr>
<tr>
<td><em>A. neotortuosum</em> Nakai</td>
<td>Unknown</td>
<td></td>
<td>N China and Korea</td>
</tr>
<tr>
<td><em>A. villosum</em> Rehb.</td>
<td>16</td>
<td>Rostovtseva, T. S. &amp; G. P. Djuragina (1977), NE China (Manchuria), Korea, Mongolia, Russia (Amur); Simon et al. (1999)</td>
<td>NE China (Manchuria), Korea, Mongolia, Russia (Amur)</td>
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<tr>
<td><em>A. volubile</em> Pall. ex Koelle</td>
<td>16</td>
<td>Yang et al. (1993); Stepanov (1994)</td>
<td>N China including Manchuria, Korea, Mongolia, Russia (Amur, Ussuri)</td>
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<td></td>
<td>32</td>
<td>Lee (2002)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>64</td>
<td>Lee (2002)</td>
<td></td>
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</tbody>
</table>

*Aconitum* subg. *Lycoctonum* (Staf.) Rapaics

<table>
<thead>
<tr>
<th>Taxon</th>
<th>Chromosome number (2n)</th>
<th>Reference</th>
<th>Distribution</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>A. albovioluteum</em> Kom.</td>
<td>16</td>
<td>Shang and Lee (1984); Yang et al. (1993); Yang (1996); Jin et al. (1998); Yuan and Yang (2006)</td>
<td>NE China, Korea, Russia (E Siberia)</td>
</tr>
<tr>
<td><em>A. longecassiatum</em> Nakai</td>
<td>16</td>
<td>Present study</td>
<td>NE China, Korea</td>
</tr>
<tr>
<td><em>A. pseudolaevig</em> Nakai*</td>
<td>16</td>
<td>Lee (1967); Present study</td>
<td>Korea (all provinces)</td>
</tr>
<tr>
<td><em>A. pteropus</em> Nakai*</td>
<td>Unknown</td>
<td></td>
<td>Korea (GR)</td>
</tr>
<tr>
<td><em>A. quelpaertesi</em> Nakai*</td>
<td>Unknown</td>
<td></td>
<td>Korea (JJ)</td>
</tr>
<tr>
<td><em>A. umbrosum</em> (Korsch.) Kom.</td>
<td>16</td>
<td>Lee (2002)</td>
<td>NE China, Korea, Russia (E Siberia)</td>
</tr>
<tr>
<td></td>
<td>16+6-2B</td>
<td>Volkova (2005)</td>
<td></td>
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</tbody>
</table>

* Endemic to Korea

* Second citation from Index to Plant Chromosome Numbers (IPCN; http://www.tropicos.org/Project/IPCN)

GB: Gyeongsangbuk-do, GN: Gyeongsangnam-do, GW: Gangwon-do, JJ: Jeju Special Self-Governing Province, JN: Jeollanam-do
in *Aconitum* for the first time from a Russian species, *A. decipiens* Vorosh., and the greatest number of B-chromosomes (up to 12) was found in *A. nagawaense* Q. E. Yang (Yang et al., 1993; Yang and Gong, 1995). Although none of the chromosome counts from Korean populations has reported B-chromosomes, they have been found in East Asian populations. B-chromosomes were reported in *A. coreanum*, *A. kasznezkii*, as well as *A. pipouneae* of Chinese populations and *A. umbrosum* of a Russian population; unstable B-chromosomes varied from 0 to 5 within/among populations (Jin et al., 1998; Yang, 2001).

In general, the *Aconitum* taxa with B-chromosomes exhibit high levels of morphological and ecological diversity (Yang et al., 1993; Jin et al., 1998).

B chromosomes of non-homologous additions can be recognized by the following features: 1) dispensable and unstable presence; 2) non-pair and non-recombine with any members of the standard A-chromosome set at meiosis; and 3) non-Mendelian and irregularity (Jones, 1975; Camacho et al., 2000; Jones and Houben, 2003). B-chromosomes have been known to be neutral in low frequency, but in high levels they potentially affect phenotypic features (Jones and Houben, 2003). Karyotypic research of taxonomically problematic species complexes in Korea, such as the *A. jaluense* complex, should be very useful for understanding hybridization and reticulation among taxa (Joachimiak et al., 1999; Jones and Houben, 2003). Further detailed cytological research is desired to understand morphological and ecological diversity of the species-rich genus, *Aconitum*.

**Acknowledgments**

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**Fig. 2.** Chromosome number variation of *Aconitum* in Korea. Chromosome numbers in *Aconitum* subgenus *Aconitum* range from 2n = 16 to 2n = 64, including 2c, 4c, and 8c with several aneuploids, whereas *Aconitum* subgenus *Lycocortonum* has only diploids (2n = 16 = 2c) (Chromosome numbers with B chromosomes are not included in the histogram). (Table 2; Fig. 2). *A. voluble* exhibits the greatest diversity from diploid to octaploid (8c), and the next diverse cytotypes are found in *A. kasznezkii*. Both species occur throughout East Asia regions from North China to Korea and belong to *Aconitum* subgenus *Aconitum*. Much greater chromosome number variation is found in the subg. *Aconitum*, which has great species diversity with ca. 250 species worldwide (more than 80% of the genus; Tamura, 1998; Li and Kadota, 2001). Chromosome numbers in subg. *Aconitum* range from 2n = 16 (2c) to 2n = 64 (8c), with several aneuploids, whereas subg. *Lycocortonum* has only diploids (2n = 16 = 2c) (Fig. 2). The latter was known to have only diploids until Yuan and Yang (2006) reported polyploidy of subg. *Lycocortonum* from the Hengduan Mountains in China. Nie et al. (2005) found comparatively low polyploidy of flowering plants (22%) and *Aconitum* (18.6%) in the Hengduan Mountains, suggesting polyploidy might have not played a major role in species diversity in the region. Unlike Yuan and Yang’s (2006) postulation of recent polyploid events of *Aconitum* in the Hengduan Mountains, those polyploids in China might be ancient. Molecular phylogeny inferred from nrDNA internal transcribed spacer (ITS) sequences and cpDNA data consider the subg. *Lycocortonum* to be an earlier lineage (Yang et al., 1993; Kita et al., 1995; Kita and Ito, 2000; Luo et al., 2005). However, Luo et al. (2005) found low genetic diversity associated with high morphological diversity in the Hengduan Mountains populations, agreeing with a previous study of Japanese populations (Kita et al., 1995). Greater chromosome number variation in subg. *Aconitum* than in subg. *Lycocortonum* suggests that chromosome number (number) evolution plays an important role for species diversity within the subgenus.
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